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MEAT EXTRACTS, THEIR COMPOSITION AND IDENTIFICATION

By JAMES A. EMERY, *Senior Biochemist*, and ROBERT R. HENLEY, *Biochemist, Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The historical aspect of meat extract has been presented so extensively in the numerous articles which from time to time have appeared in the literature that it is not considered necessary in this paper more than to refer to that phase of the question. As is well known, this product, now so generally used, owes its origin to Liebig, the chemist whose process for its preparation, as modified by Pettenkofer, has been in use in one of the large commercial houses ever since 1864.

In the method of preparation as originally described, muscle tissue alone was used for extraction, but in more recent years various influential factors, the foremost being the utilization of waste products, have caused many of the manufacturers to adapt the principles of the original process to the preparation of extracts from edible portions of the carcass other than true muscle tissue. Livers, spleens, hearts, cured-meat cook water,[1] roast-beef soak water, and bones to which more or less meat is adherent, are among the materials now employed, and the food analyst of to-day is confronted with many difficulties in his attempts to establish the identity of an extract under examination.

This investigation, therefore, was undertaken with the view of obtaining information regarding possible differences in composition of the various extracts that might be applied in formulating methods for their identification.

PREPARATION OF EXTRACTS

COMMERCIAL METHOD

Extracts of the various tissues and organs, such as chuck and plate (representing true muscle tissue), cured meat, bones (with and without adherent meat), hearts, livers, spleens, etc., were prepared, under the direct supervision of one of the authors, in the meat-extract department of one of the large commercial establishments. The method of preparation in each instance was that ordinarily used in the establishment, and

[1] Extracts were also prepared from the pickle in which the meats were cured, but the use of this material has been discontinued.

to all intents and purposes was practically the same in its essential features as that in use in the general commercial preparation of these articles. For the purpose of clarification "roast-beef soak water," "defibrinated blood," and "blood water," were added during the process of manufacture in all cases with the exception of the extracts prepared from cured meat. The comparatively large quantities employed of these agents necessarily influenced the composition of certain of the extracts, particularly those prepared from livers and spleens, and extracts of the various organs and tissues, therefore, were prepared in the laboratory, the method followed being nearly identical with the commercial process. Practically the only exception was the replacement of the materials commercially used in clarifying the extracts with those of a like composition, equally efficient, but derived from the specific tissue or organ under investigation. A detailed description of the laboratory process follows.

LABORATORY METHOD

The finely minced material from which the extract was prepared was placed in a large tin-lined box and iced water added until the minced meat was well covered. The box with its contents was then placed in the refrigerator where it was allowed to remain overnight, when the resulting "soak water" was drawn off and reserved for clarifying purposes. The partially extracted minced meat was then transferred to a large open kettle provided with a perforated steam coil, an equal weight of water added, and steam slowly applied, the temperature being gradually raised to 95° to 97° C., and the liquid kept in constant agitation by the entrance of the steam from the perforated pipe.

This extraction was continued for 45 minutes, after which the liquid was drawn off, cooled, and transferred to an evaporating kettle provided with a closed-coil steam pipe. The "soak water" obtained as above was then added, the whole brought to a boil, and the evaporation continued until the liquid was reduced to two-thirds of its original volume, the coagulable proteids which form a scum upon the surface of the liquid being removed from time to time. After this concentration the liquid was filtered and transferred to a vacuum kettle where it was evaporated under reduced pressure until the extract was of the desired consistence. This method yielded extracts identical in physical appearance and organoleptic properties with those obtained by the commercial process.

As it was also considered desirable to obtain data regarding possible differences in extracts prepared from cold and hot water extractions, the process described above was modified in the case of chuck and plate extracts prepared in the laboratory. Chuck and plate extract 29 was prepared by repeatedly exhausting the minced meat with large quantities of cold water and then concentrating the extract. Chuck and plate extract 30 was prepared by placing the minced meat in an equal

quantity of cold water, bringing the whole rapidly to a temperature of 95° to 97° C., where it was kept for 45 minutes, after which the liquor was drawn off and reduced by evaporation to the desired concentration.

It may be noted here that the two laboratory-prepared bone extracts, Nos. 27 and 28, were made by long-continued boiling of bones from which all meat had been removed.

List of extracts prepared

Commercially.	In the laboratory.
No. 10. Beef spleens.	No. 21. Beef spleens.
No. 11. Hog spleens.	No. 22. Beef spleens.
No. 12. Roast-beef soak water.	No. 23. Hog liver.
No. 13. Hog livers.	No. 24. Beef spleens.
No. 14. Bare beef bones.	No. 25. Hog liver.
No. 15. Regular bones.	No. 26. Beef hearts.
No. 16. Beef livers.	No. 27. Bones.
No. 17. Pickle.	No. 28. Bones.
No. 18. Beef hearts.	No. 29. Chuck and plate.
No. 19. Chuck and plate.	No. 30. Chuck and plate.
No. 20. Corned-beef cook liquor.	

QUANTITATIVE INVESTIGATION OF EXTRACTS

METHODS USED

In the analysis of the foregoing extracts the methods used were essentially those described by Street (*8*)[1] and, in brief, were as follows:

A 10 per cent solution of solid extract or a 20 per cent solution of liquid extract was used for the following determinations:

1. WATER.—The water representing the degree of concentration of the extract was determined by placing 20 cc. of the solution in a 100 cc. glass-stoppered weighing bottle containing 20 gm. of asbestos, and drying to constant weight in a vacuum of 30 inches at a temperature of 60° to 65° C.

2. ASH.—Ten cc. of the solution in a tared porcelain dish[2] were evaporated to dryness upon the steam bath, thoroughly carbonized at a low red heat, macerated with water, filtered, and the residue thoroughly washed and ignited. The filtrate was then added to the ignited residue in the dish, the whole evaporated to dryness upon the steam bath, ignited at a low red heat, and weighed.

3. SODIUM CHLORID.[3]—After weighing, the ash obtained was dissolved in water with the aid of a few drops of nitric acid, diluted to 100 cc., an aliquot taken, and chlorin determined by the Volhard method.[4]

[1] Reference is made by number (italic) to "Literature cited," p. 17.

[2] Porcelain was used instead of platinum in order that the possibility of volatilization of chlorin would be reduced to a minimum, as the ash was later utilized in the chlorin determination.

[3] Chlorin may be determined separately according to the method adopted by the Association of Official Agricultural Chemists. (*2*).

[4] Only a small portion of the chlorin of the ash of meat extracts is due to sodium chlorid, the greater portion being combined as chlorid of potassium (*8*). Allen (*1*) makes an allowance of 0.06 per cent sodium chlorid for every unit per cent of dry matter present, considering the excess as added salt.

4. TOTAL PHOSPHORIC ACID.—Five cc. of the solution were digested with 15 cc. each of sulphuric and nitric acids until colorless (nitric acid was added from time to time when necessary), 20 cc. of water were added, and the solution boiled in order to expel any oxids of nitrogen. It was then diluted with water, a slight excess of ammonium hydroxid added, after which it was rendered slightly acid with nitric acid, and phosphorus determined (*2*).

5. INORGANIC PHOSPHORIC ACID.—Ten cc. of the solution were diluted with from 20 to 30 cc. of water, boiled three minutes, two drops of acetic acid added, the boiling continued for a minute, cooled, and diluted to 100 cc. The solution was then filtered, a 50 cc. portion was made faintly alkaline with ammonium hydroxid, and the phosphoric acid precipitated in the usual manner with magnesia mixture. After standing for two hours or longer the precipitate was filtered off, washed with water containing 2.5 per cent of ammonia, and dissolved in dilute nitric acid. The phosphoric acid was then determined as in total phosphoric acid.

6. TOTAL NITROGEN.—Nitrogen was determined by the Gunning method, using 10 cc. of the solution.

7. SOLUBLE NITROGEN.—A portion of about 15 cc. of the solution was centrifuged until clear, the clear liquid poured off, and the nitrogen determined in a 10 cc. portion.

8. COAGULABLE NITROGEN.—Fifty cc. of the solution in a glass evaporating dish to which 50 cc. of water were added were evaporated on the steam bath to one-half volume; 0.5 cc. of a 10 per cent solution of acetic acid was added, heating was continued for 15 minutes, the coagulable albumen was filtered, washed, and nitrogen determined in the residue on the filter.

9. AMMONIA NITROGEN.—The ammonia nitrogen in these extracts was determined by the magnesium-oxid method, but the more recent and exact Folin method (*6*) is recommended.

10. NITROGEN PRECIPITATED BY ZINC SULPHATE.—Twenty-five cc. of the original solution were placed in a 50 cc. graduated flask, 1 cc. of a 50 per cent sulphuric-acid solution was added, with zinc sulphate enough to saturate the solution, after which the flask was filled to the mark with a saturated solution of zinc sulphate. After 18 hours it was filtered and the nitrogen determined by the Gunning method in 20 cc. of the filtrate, corresponding to 10 cc. of the original. The total nitrogen of the extract, less the sum of the coagulable, insoluble, and zinc-sulphate-filtrate nitrogen represents the nitrogen of the zinc-sulphate precipitate. A control determination of the nitrogen of the precipitate was also made.

11. NITROGEN PRECIPITATED BY TANNIC-ACID-SALT SOLUTION.—Twenty cc. of the original solution were placed in a 100-cc. graduated

flask, 50 cc. of a saturated sodium-chlorid solution were added, and the flask filled to the mark with a 24 per cent solution of tannic acid. After a thorough mixing it was placed in the ice box and allowed to stand overnight; any loss in volume due to contraction was corrected by the addition of the tannic-acid solution. On the following day it was filtered, the solution being kept in the ice box during filtration, and 50 cc. of the filtrate, corresponding to 10 cc. of the original, were transferred to a Kjeldahl flask and evaporated to dryness on the steam bath with the aid of a current of air. The nitrogen in the dried residue was determined by the Gunning method and control determinations made on the reagents used.

Nitrogen in the tannic-acid-salt precipitate was obtained by subtracting the sum of the tannic-acid-salt filtrate and the coagulable and insoluble nitrogen from the total nitrogen.

12. "MEAT-BASE" NITROGEN.—This was obtained by subtracting the sum of the coagulable, insoluble, ammonia, and tannic-acid-salt precipitate nitrogen from the total nitrogen.

13. NITROGEN DUE TO PEPTONE-LIKE BODIES.—This was found by deducting the proteose nitrogen obtained by precipitation with zinc sulphate from the total quantity of nitrogen precipitated by the tannic-acid-salt reagent.

14. NONNITROGENOUS ORGANIC MATTER.—This was determined by difference. From the ash-free total solids was deducted the sum of the products of the "meat-base" nitrogen × 3.12 and the nonmeat-base nitrogen × 6.25.

15. PURINS (*3*).—Three gm. of the sample were dissolved in 500 cc. of a 1 per cent solution of sulphuric acid and heated for four hours in an open dish on the steam bath. (At the end of this time about 75 cc. should remain.) It was then neutralized with caustic soda, with litmus paper as an indicator, transferred to a beaker, and 15 cc. of a 15 per cent solution of sodium bisulphite and 15 to 20 cc. of a 15 per cent solution of copper-sulphate solution were added. This was allowed to stand overnight, filtered, washed with dilute copper-sulphate solution, and the precipitate then washed with hot water from the paper into the original beaker. The contents of the beaker were brought to the boiling point and sodium sulphid added to precipitate all of the copper. It was then placed upon the steam bath for several minutes, made acid with acetic acid, and allowed to settle thoroughly, after which the precipitate was filtered off, washed with hot water, 10 cc. of 10 per cent hydrochloric acid added to the filtrate washings, and the solution evaporated to dryness on the steam bath. Ten cc. more of 10 per cent hydrochloric acid were added and digestion was continued until the bases in the residue were dissolved. It was then filtered, washed, the filtrate made alkaline with 25 cc. of concentrated ammonium hydroxid, 10 cc. of a 3 per cent

ammoniacal silver-nitrate solution added, allowed to stand overnight, filtered on the following morning, the residue on the paper washed until all traces of ammonia were removed, and its nitrogen content determined.

16. CREATININ.—The method of Folin as modified by Emmett and Grindley (5) was used. An aliquot free from coagulable and insoluble nitrogen and containing from 7 to 15 mgm. of creatinin was placed in a 500 cc. flask, 15 cc. of picric acid and 10 cc. of a 10 per cent solution of sodium hydrate added, allowed to stand for five minutes, being agitated several times in the interim, and then diluted to 500 cc. After mixing, a portion of the solution was poured into one tube of a Duboscq colorimeter and compared with *N/2* potassium-bichromate solution contained in the other tube, the scale of which was set at 8.0.

Creatinin was calculated by the following formula:

$$\left\{\frac{8.1}{\text{Reading}} \times \frac{\text{Volume}}{500}\right\} \times 10 = \text{milligrams of creatinin in the aliquot taken.}$$

17. CREATIN.—To 5 cc. of the extract in a 50 cc. graduated flask, 10 cc. of *N/1* hydrochloric acid and 5 cc. of water were added, and the solution heated in an autoclave at 135° C. for 30 minutes. It was then cooled, 10 cc. of *N/1* sodium hydroxid added and the solution made to volume with water. An aliquot was taken and creatinin determined as above, with 30 cc. of 1.2 per cent picric acid and 10 cc. of a 10 per cent solution of sodium hydroxid as suggested by Emmett and Grindley (5), the result so obtained representing the total creatinin—creatinin due to creatin and to preformed creatinin. The difference between the total creatinin and the preformed creatinin multiplied by 1.16 represents the creatin.

18. NITRATES.—To a few drops on a porcelain spot plate of a reagent containing 0.1 to 0.2 gm. of diphenylamin (4) in 100 cc. concentrated sulphuric acid were added a few drops of the extract solution. In the presence of nitrates a blue color developed. They were then quantitatively estimated by the Schlossing-Wagner method (9).

DISCUSSION OF QUANTITATIVE RESULTS

The results of the quantitative chemical examination of the extracts are presented in Table I and, calculated to a water-free basis, in Table II. In Table III differences in the forms of nitrogen are shown. The percentages of creatin and creatinin appear in Table IV together with the ratio between total nitrogen and the sum of the creatin and creatinin. The percentages in this table are also calculated on a water-free basis.

TABLE I.—*Results of chemical examination of meat extracts*

COMMERCIAL EXTRACTS

Extract No.	Water.	Total solids.	Ash.	Organic matter.	Nonnitrogenous organic matter.	Chlorin.			Phosphorus.			Acidity.		Nitrogen.											Preformed creatinin.	Creatin.	Nitrates.
						Chlorin.	Sodium chlorid (chlorin × 1.65).	Natural chlorids (total solids × 0.06).[a]	Total phosphorus pentoxid.	Inorganic phosphorus pentoxid.	Ratio of inorganic to total.	*N*/10 sodium hydroxid to 100 gm. of extract.		Total.	Insoluble.	Coagulable.	Ammonia.	Zinc sulphate precipitate.	Tannic-salt precipitate.	Peptone-like bodies.	Meat bases.								
												Litmus.	Phenolphthalein.								Total (by difference).	Creatin.	Preformed creatinin.	Purins.	Undetermined.			
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Cc.*	*Cc.*	*Per cent.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per ct.*	*Per cent.*	*Per ct.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
10. Beef spleens	23.29	76.71	20.39	56.32	24.96	3.87	6.45	4.60	3.10	2.17	0.700	[b] A	720	7.66	0.14	0.00	0.26	2.30	3.98	1.68	3.28	0.21	0.48	0.69	1.90	1.31	0.68	None.
11. Hog spleens	19.46	80.54	23.01	57.53	20.55	3.78	6.30	4.83	5.17	3.68	.711	A	756	7.56	.00	.06	.29	2.01	4.05	2.04	3.16	.18	.44	.80	1.74	1.21	.59	None.
12. Roast-beef soak water	11.70	88.30	33.64	54.66	17.49	7.11	11.85	5.29	6.40	5.84	.902	A	1,121	7.94	.08	.00	.35	.82	3.53	2.71	3.98	.25	2.27	.59	.87	6.13	.79	None.
13. Hog livers	19.00	81.00	20.78	60.22	36.47	4.96	8.28	4.86	4.67	2.93	.629	295	983	4.85	.01	.05	.09	.91	2.60	1.69	2.10	.16	.47	.52	.95	1.29	.50	None.
14. Bare beef bones	16.10	83.90	29.55	54.35	17.06	5.55	9.25	5.03	5.53	4.98	.900	A	942	7.95	.10	.00	.23	1.13	3.98	2.85	3.64	.37	1.92	.58	.67	5.18	1.16	None.
15. Regular bone	17.00	83.00	28.62	54.28	19.05	5.40	9.00	4.98	5.14	4.56	.904	A	857	7.96	.16	.02	.23	1.20	3.55	2.35	4.00	.42	2.02	.57	.99	5.46	1.32	None.
16. Beef livers	18.18	81.82	21.76	60.06	39.98	4.86	8.11	4.91	5.25	3.31	.630	695	1,192	5.40	.03	.06	.09	1.70	3.02	1.32	2.20	.23	.46	.51	1.00	1.26	.73	None.
17. Pickle	11.10	88.90	27.50	61.40	31.95	5.62	9.37	5.33	1.52	1.39	.914	509	1,437	6.66	.00	.05	.27	.76	2.45	1.69	3.89	.07	1.14	.38	2.30	3.09	.25	14.45
18. Beef hearts	21.30	78.70	24.48	54.22	22.00	4.57	7.63	4.72	5.49	4.64	.845	A	1,046	7.10	.06	.00	.40	1.23	2.82	2.56	3.88	.32	1.34	.65	1.48	3.65	1.00	None.
19. Chuck and plate	19.80	80.20	23.20	57.00	19.43	3.23	5.39	4.81	6.16	5.18	.844	493	1,073	8.09	.01	.06	.30	1.44	3.57	2.13	4.15	.51	1.69	.57	1.38	4.57	1.61	None.
20. Corned-beef cook liquor	17.00	83.00	23.78	59.22	22.43	5.27	8.79	4.98	1.87	1.42	.760	197	809	7.66	.08	.06	.50	1.32	3.16	1.84	3.86	.36	1.24	.58	1.68	3.34	1.13	8.70

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21. Beef spleens	24.78	75.22	19.22	57.00	17.36	2.58	4.33	4.51	5.36	.....	.....	A	815	7.51	0.10	0.14	0.74	1.68	4.21	2.53	2.32	0.00	0.06	0.91	1.35	0.18	0.01	None.
22. Beef spleens	28.09	71.91	16.57	55.34	16.71	1.91	3.19	4.31	5.22	4.19	0.802	A	825	7.75	.17	.00	.81	1.74	3.64	1.90	3.13	.00	.06	1.08	1.99	.17	.01	None.
23. Hog liver	19.75	80.25	21.36	58.89	24.67	2.50	4.17	4.80	7.75	4.45	.575	98	790	6.53	.00	.06	.59	1.58	3.84	2.26	2.04	.03	.08	.25	1.68	.22	.03	None.
24. Beef spleens	30.08	69.92	20.29	49.63	16.56	3.08	5.14	4.19	5.41	3.64	.672	A	651	6.36	.00	.40	.30	1.33	3.53	2.20	2.13	.00	.08	.43	1.62	.22	.02	None.
25. Hog liver	19.36	80.64	15.98	64.66	39.55	2.15	3.59	4.80	10.59	3.35	.317	251	929	5.26	.04	.07	.24	.60	2.75	2.15	2.16	.01	.07	.29	1.79	.19	.03	None.
26. Beef hearts	19.91	80.09	23.24	56.85	25.05	2.66	4.44	4.80	6.52	5.57	.854	279	1,058	7.04	.12	.00	.83	.78	2.15	1.37	3.93	.47	1.65	.57	1.25	4.16	1.28	None.
27. Bones [c]	57.35	42.65	8.09	33.56	13.21	1.78	2.97	2.52	.71	.63	.887	59	59	3.52	.18	1.46	.09	.54	1.26	.72	.53	.00	.22	.05	.26	.59	.00	None.
28. Bones [c]	44.97	55.03	18.86	36.17	10.25	5.50	9.18	3.80	.81	.46	.567	26	.....	4.64	.00	.09	.18	2.58	3.57	.99	.90	.01	.19	....	.70	.53	.04	None.
29. Chuck and plate [d]	11.63	88.37	27.13	61.24	23.72	2.52	4.20	.....	6.78	.....	.....	889	1,581	8.57	....	.03	.70	.....	2.39	.....	5.45	.11	2.41	.68	2.25	6.48	.37	None.
30. Chuck and plate [e]	17.21	82.79	23.48	59.31	12.14	2.03	3.39	.....	6.11	4.82	.788	348	946	9.66	....	.05	.65	2.09	4.73	2.64	4.23	.91	.91	.70	1.71	2.46	2.84	None.

[a] See Allen (1, p. 307).
[b] Amphoteric.
[c] These extracts gave a very cloudy solution, muddy-like, and had an offensive soapy odor.
[d] Cold-wa er extract; extracted four times with cold water, then boiled and concentrated.
[e] Hot-water extract; placed in tcold water and immediately brought to a boil.

TABLE II.—*Results of chemical examination of meat extracts (water-free basis)*

COMMERCIAL EXTRACTS.

Extract No.	Ash.	Organic matter.	Nonnitrogenous organic matter.	Chlorin.		Phosphorus.			Nitrogen.													Creatinin.	Creatin.
																Meat bases.							
				Chlorin.	Sodium chlorid (Chlorin×1.65).	Total phosphorus pentoxid.	Inorganic phosphorus pentoxid.	Ratio of inorganic to total.	Total.	Insoluble.	Coagulable.	Ammonia.	Zinc-sulphate precipitate.	Tannic-salt precipitate.	Peptone-like bodies.	Total (by difference).	Creatin.	Creatinin.	Purins.	Undetermined.		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
10. Beef spleens	26.59	73.41	24.07	5.04	8.41	4.04	2.83	0.700	9.98	0.18	0.00	0.34	3.00	5.17	2.17	4.16	0.27	0.62	0.88	2.47	1.70	0.88
11. Hog spleens	28.58	71.42	25.23	4.70	7.83	6.42	4.57	.711	9.38	.00	.07	.36	2.49	4.92	2.43	3.91	.22	.54	.99	2.14	1.50	.73
12. Roast-beef soak water	38.10	61.90	19.70	8.05	13.42	7.25	6.62	.902	8.99	.09	.00	.39	.92	3.96	3.04	4.49	.28	2.58	.67	.98	6.94	.89
13. Hog livers	25.65	74.35	44.96	6.12	10.22	5.76	3.62	.629	6.00	.03	.05	.11	1.12	3.20	2.08	2.59	.20	.58	.65	1.16	1.59	.61
14. Bare beef bones	35.20	64.80	19.17	6.60	11.02	6.55	5.94	.900	9.47	.12	.00	.28	1.34	4.73	3.39	4.33	.44	2.28	.69	.79	6.18	1.38
15. Regular bones	34.48	65.52	21.62	6.50	10.84	6.07	5.49	.904	9.59	.20	.00	.29	1.44	4.27	2.83	4.80	.50	2.42	.68	1.18	6.60	1.59
16. Beef livers	26.58	73.42	40.62	6.01	10.03	6.42	4.05	.630	6.59	.03	.07	.11	2.07	3.68	1.61	2.68	.28	.56	.62	1.22	1.54	.89
17. Pickle	30.92	69.08	29.30	6.32	10.54	1.76	1.56	.914	7.60	.....	.02	.30	.86	2.64	1.78	4.35	.08	1.28	.42	2.57	3.48	.28
18. Beef hearts	31.10	68.90	29.74	5.82	9.70	6.98	5.90	.845	9.02	.08	.00	.49	1.55	3.61	2.06	4.84	.40	1.70	.77	1.89	4.63	1.26
19. Chuck and plate	28.90	71.10	24.20	4.03	6.72	7.66	6.46	.844	10.08	.00	.08	.37	1.79	4.45	2.66	5.14	.63	2.11	.70	1.71	5.69	2.00
20. Corn-beef cook liquor	28.65	71.35	28.21	6.35	10.59	2.25	1.71	.760	9.23	.10	.07	.60	1.59	3.79	2.20	4.63	.73	1.48	.70	2.01	4.02	1.36
LABORATORY EXTRACTS																						
21. Beef spleens	25.56	74.44	24.79	3.45	5.75	7.12	.....	.....	9.98	0.13	0.18	0.98	2.37	5.60	3.23	3.08	0.00	0.07	1.21	1.80	0.23	0.01
22. Beef spleens	23.03	76.97	23.51	3.25	5.43	7.25	5.82	0.803	10.77	.23	.00	1.13	2.41	4.82	2.41	4.35	.00	.08	1.50	2.77	.23	.01
23. Hog livers	26.51	73.49	29.54	3.11	5.19	9.65	5.54	.575	8.14	.00	07	.73	1.97	4.75	2.78	2.53	.00	.10	.31	2.11	.39	.04
24. Beef spleens	29.01	70.99	24.11	4.47	7.45	7.73	5.19	.679	9.07	.00	.56	.42	1.91	5.04	3.13	3.35	.00	.11	.61	2.63	.31	.03
25. Hog livers	19.81	80.19	48.79	2.67	4.45	13.13	4.15	.316	6.52	.05	.09	.29	.61	3.41	2.80	2.68	.00	.08	.36	2.23	.24	.04
26. Beef hearts	28.98	72.02	32.57	3.31	5.53	8.13	6.93	.854	8.77	.14	.00	1.03	.98	2.69	1.71	4.91	.53	.05	.71	1.62	6.64	1.60
27. Bones	18.90	78.76	31.06	4.18	6.97	1.76	1.48	.887	8.26	.43	3.42	.18	1.26	1.66	1.82	1.24	.00	.51	.11	.61	1.12	.00
28. Bones	33.98	65.19	18.00	10.43	15.73	1.46	.83	.561	8.36	.00	.16	.32	4.81	6.44	1.63	1.62	.....	.34	.....	1.26	1.02	.07
29. Chuck and plate	30.68	69.32	28.02	2.85	4.75	7.67	.....	.....	9.69	.....	.04	.79	.....	2.70	.....	6.16	.13	2.73	.77	2.54	7.32	.41
30. Chuck and plate	28.36	71.64	14.70	2.44	4.09	7.38	5.82	.788	11.67	.....	.06	.78	2.52	5.71	3.19	5.11	1.27	1.10	.84	2.06	2.97	3.43

TABLE III.—*Distribution of nitrogen in meat extracts*

Extract No.	Method of preparation.	Non-nitrogenous matter.	Total nitrogen.	Total nitrogen in—		
				Zinc-sulphate precipitate.	Tannic-salt precipitate.	"Meat base."
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
19. Chuck and plate	Commercial	24.20	10.08	17.75	44.13	50.98
29. Chuck and plate	Laboratory	28.02	9.69	.....	27.86	63.56
30. Chuck and plate	do	14.70	11.67	21.57	48.87	43.74
12. Roast-beef soak water	Commercial	19.70	8.99	10.34	44.05	49.94
20. Corn-beef cook liquor	do	28.21	9.23	17.21	41.04	50.13
14. Beef bones	do	19.17	9.47	13.58	47.96	43.90
15. Beef bones	do	21.62	9.59	15.00	44.49	50.01
17. Pickle	do	29.30	7.60	11.31	34.71	57.20
18. Beef hearts	do	29.74	9.02	17.17	39.99	53.62
26. Beef hearts	Laboratory	32.57	8.77	11.17	30.76	55.97
10. Beef spleens	Commercial	24.07	9.98	30.07	51.81	41.68
11. Hog spleens	do	25.23	9.38	26.53	52.45	41.68
21. Hog spleens	Laboratory	24.79	9.98	23.74	56.10	30.87
22. Hog spleens	do	23.51	10.77	22.36	44.63	40.37
24. Hog spleens	do	24.11	9.07	21.14	55.54	36.91
13. Hog liver	Commercial	44.96	6.00	18.66	53.33	43.16
16. Beef liver	do	40.62	6.42	32.23	57.29	41.72
23. Hog liver	Laboratory	30.54	8.14	24.19	58.37	31.06
25. Hog liver	do	48.79	6.52	9.35	52.30	41.10
Averages:						
Chuck and plate, bones, liquors		22.23	9.81	15.90	42.63	50.32
Hearts		31.65	8.89	14.17	35.33	54.79
Spleens		24.34	9.81	24.76	52.10	38.30
Livers		41.22	6.77	21.10	55.32	39.26

In consulting these tables it will be noted that the percentage quantities of certain constituents show marked and characteristic differences, depending upon the nature of the extract. The most striking variations are the figures representing total nitrogen, "meat-base" nitrogen, creatinin, and nonnitrogenous organic matter. Differences in the amounts of the other constituents, with the exception of the ratio of total phosphorus to inorganic phosphorus, are not considered sufficiently marked to justify their being used, and attention is directed to the following results:

1. TOTAL NITROGEN.—This was found to be very low in liver extracts, as compared with other extracts. The percentage of total nitrogen in one of the liver extracts (No. 23) is much higher than that of the remaining three, but is, nevertheless, lower than that of any of the other extracts with the exception of the pickle extract. Chuck and plate extracts contain the largest quantity of nitrogen, with spleen extracts next. The other extracts vary between 9 and 10 per cent total nitrogen with the exception of the pickle extract, which is very low (7.60 per cent).

TABLE IV.—*Distribution of creatin and creatinin (water-free basis)*

Extract No.	Method of preparation.	Total nitrogen.	Creatin.	Creatinin.	Total creatin plus creatinin.	Ratio of creatin plus creatinin to nitrogen.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
19. Chuck and plate	Commercial	10.08	2.00	5.69	7.69	0.763
29. Chuck and plate	Laboratory	9.69	.41	7.32	7.73	.797
30. Chuck and plate	do	11.67	3.43	2.97	6.40	.463
12. Roast-beef soak water	Commercial	8.99	.89	6.94	7.83	.870
20. Corn-beef cook liquor	do	9.23	1.36	4.02	5.38	.583
14. Beef bones	do	9.47	1.38	6.18	7.56	.798
15. Beef bones	do	9.59	1.59	6.60	8.19	.854
17. Pickle	do	7.60	.28	3.48	3.76	.493
18. Beef hearts	do	9.02	1.26	4.63	5.89	.653
26. Beef hearts	Laboratory	8.77	1.60	6.64	8.24	.939
10. Beef spleens	Commercial	9.98	.88	1.70	2.58	.258
11. Hog spleens	do	9.38	.73	1.50	2.23	.237
21. Hog spleens	Laboratory	9.98	.01	.23	.24	.024
22. Hog spleens	do	10.77	.01	.23	.24	.022
24. Hog spleens	do	9.07	.03	.31	.34	.037
13. Hog liver	Commercial	6.00	.61	1.59	2.20	.366
16. Beef liver	do	6.42	.89	1.54	2.43	.368
23. Hog liver	Laboratory	8.14	.04	.39	.43	.053
25. Hog liver	do	6.52	.04	.24	.28	.043
Averages:						
Chuck and plate, bones, liquors		9.81	.....	.....	7.25	.732
Hearts		8.89	.....	.....	7.06	.796
Commercial spleens		9.68	.....	.....	2.40	.248
Laboratory spleens		9.94	.....	.....	.27	.031
Commercial livers		6.21	.....	.....	2.31	.367
Laboratory livers		7.33	.....	.....	.36	.048

2. NONNITROGENOUS ORGANIC MATTER.—As will be noted in Table III, liver extracts, as compared with the other extracts, are extraordinarily high in nonnitrogenous organic matter, containing, with the exception of extract 23, more than 40 per cent. Heart extracts (containing 30 per cent) more nearly resemble liver extracts.

3. "MEAT-BASE" NITROGEN.—The determination of total nitrogen and of "meat-base" nitrogen enables one with a fair degree of certainty to differentiate liver extracts and spleen extracts from each other and from other extracts as well. While liver extracts and spleen extracts differ from other extracts in showing a low percentage of "meat-base" nitrogen, they differ from each other in that the spleen extracts show a high total nitrogen, whereas the total nitrogen of liver extracts is low.

In liver extracts the "meat-base" nitrogen constitutes only about 40 per cent of the total nitrogen, while in other extracts, with the exception of spleen extracts, the figure is nearer 50 per cent. Particular attention is called to liver extract 23, which contains 8.14 per cent total nitrogen and pickle extract 17, containing 7.60 per cent total nitrogen. Although the pickle extract contains much less total nitrogen than the liver extract, a much larger proportion of this nitrogen (57.23 per cent) is "meat-base"

nitrogen. In the liver extract the "meat-base" nitrogen constitutes only 31.08 per cent of the total nitrogen.

Chuck and plate extract 30 is much lower in "meat-base" nitrogen than the other chuck and plate extracts. This is exceptional and is undoubtedly due to the laboratory process used in its preparation, the extraction having been made entirely with hot water. (See p. 2.)

4. PROTEOSE NITROGEN (zinc-sulphate precipitate, Table III).—Although the quantity of the proteose nitrogen varies from 9.35 to 32.23 per cent of the total nitrogen, the amounts in any one kind of extract are not sufficiently constant to render the figure of any value in the identification of extracts. On the whole, however, liver and spleen extracts are somewhat higher in that constituent than other extracts. This factor is probably influenced more by the process used in the preparation of the extract than by the material from which the extract is made.

5. CREATIN AND CREATININ.—It is in the total creatinin content of the various extracts that the greatest and most uniform differences occur. The sum of the quantities of creatin and creatinin, together with the ratio between this total and the total nitrogen of the extracts, is shown in Table IV. It appears from these results that a determination of the total creatinin will suffice in any case to classify an extract, if pure, as a liver or spleen extract, on the one hand, or as a true meat extract on the other.[1]

It will be noticed at once that the liver and spleen extracts prepared under commercial conditions contain about 10 times as much total creatinin as the laboratory extracts, attributable to the creatinin of the roast-beef soak water, defibrinated blood, and blood water used in clarifying these extracts. However, even though these commercially prepared liver and spleen extracts are relatively high in creatinin, they are, nevertheless, much lower than any of the other extracts. The greatest quantity of creatinin found in any of the liver and spleen extracts is 2.58 per cent and the highest total creatinin—total nitrogen ratio—0.37, while the smallest amount of creatinin in the other extracts (except the pickle extract) is 5.38 per cent and the lowest ratio 0.46. From these results it appears that all extracts of fresh flesh, with the exception of extracts of liver and spleen, contain more than 5 per cent of total creatinin.

6. PHOSPHORUS.—Rather marked differences occur in the amounts of phosphorus found in the extracts as well as in the relation existing between the inorganic and total phosphorus present.

The extracts of pickle and of corned-beef cook liquor contain very little phosphorus, about 2 per cent; none of the other extracts contain less than 5 per cent with the exception of spleen extract 10. The laboratory liver extracts are noticeably high in phosphorus.

[1] Extracts from bones as made commercially will show a relatively high total creatinin. This is due to the fact that the commercial bone extracts are essentially meat extracts, most of the extractives in them being derived from the adherent meat and the clarifying agents which are used, and not from the bones themselves.

Differences which are highly characteristic are found in the relation of total and inorganic phosphorus, the ratio of inorganic phosphorus pentoxid to total phosphorus pentoxid being much lower for liver than for other extracts, the next higher being that of spleens. Grouping these ratios, livers have a ratio lower than 0.65; spleens a ratio near 0.7; hearts, chuck, and plate, and corned-beef cook liquor run above 0.75 and nearer 0.8; and the ratio in the remaining extracts is 0.90 or higher.

SUMMARY OF QUANTITATIVE DIFFERENCES

Liver extracts are low both in total nitrogen and "meat-base" nitrogen; have a low inorganic phosphorus to total phosphorus ratio, are very low in total creatinin, and as a rule are very high in nonnitrogenous organic matter.

Spleen extracts are high in total nitrogen, low in "meat-base" nitrogen, very low in creatinin, and lower than other extracts, liver excepted, in the inorganic-phosphorus to total-phosphorus ratio.

Heart extracts are low in total nitrogen as compared with chuck and plate extracts, but much higher than liver. They contain considerable nonnitrogenous organic matter, being next to liver extracts in this respect. Heart extracts differ from liver and spleen extracts in total creatinin and in "meat-base" nitrogen, the latter comprising at least 50 per cent of the total nitrogen in heart extracts.

Pickle and cured-meat extracts are readily identified by the presence of nitrates, which are always present in such extracts. The quantity of total phosphorus present in such extracts is very small. In other respects cured-meat extracts are found to resemble true-meat extracts. Pickle extracts contain rather less creatinin than true-meat extracts.

Chuck and plate extracts run high in total nitrogen, "meat-base" nitrogen, and total creatinin and have a high inorganic-phosphorus to total-phosphorus ratio.

The bone extracts prepared commercially [1] and the extract prepared from roast-beef soak water resemble chuck and plate extract.

PHYSICAL CHARACTERISTICS OF EXTRACTS

In addition to the chemical differences which have been discussed above marked physical characteristics of the extracts exist which in many cases are so decided that workmen engaged in their manufacture become very expert in identifying meat extracts solely by their physical appearance. The properties upon which their judgment is based are color, texture, and "shortness," an extract being termed "short" when it quickly and easily breaks upon testing its elasticity.

[1] The bone extracts prepared in the laboratory have not been discussed, as they do not in the least resemble commercial bone extracts, and are included in Tables I and II merely as a matter of general interest.

Liver extracts are very dark brown, almost black, in color, are very gummy—that is, they are not "short," and their solution in water is dark red, with a trace of fluorescence.

Spleen extracts are light-chocolate to light yellow-brown in color have a smooth texture, and are very "short."

Bone extracts closely resemble spleen extracts.

Other extracts, including heart extract, are darker than spleen extract, but not so dark as liver extract. They are usually very "short," and their solutions are dark, but are not fluorescent.

QUALITATIVE INVESTIGATION OF EXTRACTS

In addition to the quantitative differences in extracts qualitative differences have been noted and, based on these differences, qualitative tests have been devised for the identification of liver and spleen extracts either when pure or in the absence of any considerable proportions of true-meat extracts. In mixtures in which liver or spleen extracts are present in only small amounts the tests are not entirely dependable, although in such instances they are as reliable as any other known method.

ACETIC-ACID TEST

A qualitative test for the identification of spleen extracts was suggested by Robert M. Chapin, of the Biochemic Division, who noticed that the addition of an excess of acetic acid to a spleen extract resulted in the formation of an abundant precipitate. Confirmation of this observation was found in the literature, Hammarsten (7) stating that spleens are characterized by a peculiar protein which is soluble in boiling water, but which is precipitated by an excess of acetic acid.

Acting upon this information, the writers tested all the commercially prepared spleen extracts (the laboratory-prepared extracts having been exhausted in the quantitative investigation) with acetic acid in the manner described below.

About 30 cc. of a 10 per cent solution of the extract under examination are boiled, filtered, the filtrate cooled, and an equal quantity of a 10 per cent solution of acetic acid added.

Extract No.	Effect of acetic acid.
10. Beef spleens	A dense, white precipitate.
11. Hog spleens	A dense, white precipitate.
12. Roast-beef soak water	No effect.
13. Hog livers	A slight, dark precipitate.
14. Bare beef bones	No effect.
15. Regular bones	No effect.
16. Beef livers	A slight, dark precipitate.
18. Beef hearts	No effect.
19. Chuck and plate	No effect.
20. Corned-beef cook liquor	A very slight yellowish precipitate.

With spleen extracts only was a decided precipitate obtained. The precipitate was very bulky and yellowish white in color, easily distinguishable from both the slight, dark precipitate yielded by the liver extracts and the slight precipitate obtained with the corned-beef cook liquor.

Mixtures of varying amounts of hog spleens (No. 11) and chuck and plate extract (No. 19) were prepared and tested with acetic acid in the manner shown above, in order to determine the delicacy of the reaction. A precipitate was obtained in mixtures containing 5 per cent of spleen extract, but the reaction was faint, and it was not until the mixed extract contained a quantity approximating 20 per cent that a decided reaction was obtained.

Since the above tests were made an extract has been received and examined under the meat-inspection regulations which gave a positive reaction with the acetic-acid test, although not resembling spleen extracts in other respects.

An investigation by the field service of the Meat Inspection Division disclosed that this extract was prepared from bones through long-continued extraction with boiling water. Inasmuch as bone extract previously examined had not given the reaction, a laboratory investigation of this unusual feature was made, three bone extracts being prepared by extraction with boiling water for at least three hours. Upon testing the finished products the reaction, which heretofore had been limited to spleen extracts, was obtained. The precipitate from both the spleen and these bone extracts appeared to be a mucin. From the standpoint of the food analyst the value of the test, however, is not lessened, as the differentiation of bone extracts from other extracts, including spleen, is readily made, which may be noted by comparing the analyses given in Table V with those of other extracts previously tabulated (see Table II).

TABLE V.—*Analyses of bone extracts*

Constituent.	Extract 1.	Extract 2.	Extract 3.
	Per cent.	*Per cent.*	*Per cent.*
Total solids	57.54	62.90	63.53
Ash	3.42	13.80	14.81
Sodium chlorid	.81	7.58	7.32
Phosphorus pentoxid (total)	.29	.49	.59
Nitrogen	8.54	7.90	10.40
Tannic-acid salt filtrate nitrogen	.35	1.46	1.37
Zinc-sulphate filtrate nitrogen	3.23	2.21	3.60
Total creatinin	None.	None.	None.
Acetic-acid test	Positive.	Positive.	Positive.
Molisch test	Negative.	Negative.	Negative.

While not applying to the real purpose of this paper, it may be of interest to note that an extract of bone marrow failed to afford a precipitate with acetic acid.

MOLISCH TEST

By determining the total creatinin content of an extract, the water content being known, it can be definitely classified as either a true-meat extract or as an extract of liver or spleen, and further, an extract identified as having been prepared from either livers or spleens may be further classified as either a spleen or a liver extract, depending upon the reaction in the acetic-acid test. While the identity of a liver extract may thus be established, qualitative tests confirmatory of the conclusions arrived at through the quantitative examination were applied. A reaction based on the occurrence in liver extracts of comparatively large amounts of carbohydrates was suggested and the Molisch test was employed.

METHOD OF USING MOLISCH TEST.—One cc. of a 10 per cent solution of solid or of a 20 per cent solution of fluid extract was placed in a graduated glass-stoppered cylinder of 25 cc. capacity, 9 cc. of concentrated sulphuric acid were allowed to flow gently down the sides of the cylinder, and 6 to 10 drops of a 20 per cent alcoholic solution of alphanaphthol were then added. The stopper was inserted, and the contents of the cylinder were thoroughly mixed. In the presence of carbohydrates a persistent and intense reddish-purple to deep-violet color developed immediately.

In the initial tests the color of the mixture in the cylinder was noted one minute after shaking, and the contents of the cylinder were then poured into 200 cc. of water, the color again being observed. It was later found that a more satisfactory color test was obtained when the mixture in the cylinder was allowed to stand overnight and observed directly.

Extracts of known origin were tested with the results given in Table VI.

TABLE VI.—*Results of Molisch test of meat extracts of known origin*

Extract.	Method of preparation.	Color on shaking.[a]	Color in water.[a]	Color after standing.[a]
Roast-beef soak water	Commercial	No color	No color	No color.
Beef bones	do	do	do	Do.
Beef chuck	do	do	do	Do.
Beef spleens	do	Very faint purple.	Very faint purple.	Do.
Hog spleens	do	do	do	Do.
Beef liver	do	Strong	Strong	Strong.
Do	do	Pronounced	Pronounced	Pronounced.
Beef chuck	Laboratory	Very faint	Very faint	No color.
Hog liver	Commercial	Pronounced	Pronounced	Pronounced.
Beef melts (spleens)	Laboratory	Very faint	Very faint	Very faint.
Beef liver	do	Pronounced	Pronounced	Pronounced.
Do	do	do	do	Do.
Do	do	do	do	Do.

[a] In cases marked "no color" there was no trace of the characteristic purple-red color, but there was usually a greenish-brown color.

Mixtures of liver extracts with chuck and plate extracts in varying proportions were prepared for the purpose of determining the delicacy of the reaction, and it was found that the characteristic color was recognizable in an extract containing 20 per cent of liver extract; smaller quantities gave a purple color, but it was not sufficiently distinct to be of value.

In all cases where a positive reaction is obtained and in which the other factors, such as creatinin, nitrogen, etc., indicate the absence of liver extract, the sample should be examined for starch and cane sugar.

It may be stated that, aside from its value in indicating the presence of liver extract, the Molisch test is necessary in a routine examination for the rapid detection of carbohydrates which may have been added to meat extracts.[1] In the analyses of some hundreds of extracts a true-meat extract has never yielded a positive reaction with this test, and whenever a positive reaction is obtained in an extract which can be shown to contain no liver extract it is due to added carbohydrate. In such instances the test should be supplemented with a more complete examination to identify the carbohydrate thus indicated.

COPPER TEST

During the course of the investigation it was also noted that the ash of liver extracts in every instance exhibited a more or less pronounced greenish color, which was not observed in the ash of any of the large number of other kinds of extracts examined. As the presence of copper in livers has been demonstrated, it having been found even in the liver of the fetus, the presence of copper in the ash of extracts other than those prepared from livers should be considered. As practically all the commercial extracts prepared from other tissues and organs underwent the same course of preparation, and no copper was thus indicated in the ash of any of the resulting products, it would seem that this test would apply alone to the ash of extracts obtained from livers. If, however, copper utensils are used in the preparation of an extract its presence in limited amounts could probably be readily demonstrated by chemical methods, but a greenish-tinted ash should always lead one to suspect the presence of liver extract.

Procedure suggested in the identification of an extract:

1. Total solids.
2. Ash.
3. Sodium chlorid.
4. Total phosphoric pentoxid.
5. Inorganic phosphoric pentoxid.
6. Total nitrogen.
7. "Meat-base" nitrogen.
8. Preformed creatinin.
9. Creatin.
10. Molisch test.
11. Acetic-acid test.
12. Test for starch and sugar if a positive Molisch test is given.
13. Test for nitrates.
14. Test of ash for copper.

[1] As an illustration of the value of this test, regardless of its value in detecting the presence of liver, several extracts have been examined which conformed in every respect to pure-meat extracts with the exception that they gave a positive Molisch test. Upon investigation the presence of sucrose was demonstrated.

The quantities of insoluble, coagulable, and ammonia nitrogen are so small in all ordinary extracts that they are determined only in case they are indicated in extraordinary amounts. Should an extract show an unusually high nitrogen content a study of the various forms of nitrogen present is essential.

After an examination of an extract as suggested its accurate classification as a true-meat extract, as a cured-meat extract, as a compound extract, or as an extract of liver or spleen is possible, and at the same time the addition of foreign material, such as sugar, starch, or salt, will be established.

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QUANTITY AND COMPOSITION OF EWES' MILK: ITS RELATION TO THE GROWTH OF LAMBS

By RAY E. NEIDIG, *Chemist*, and E. J. IDDINGS, *Dean and Director, Idaho Agricultural Experiment Station*[1]

INTRODUCTION

During the progress of an investigation upon different breeds of sheep at the Idaho Agricultural Experiment Station observations were made upon the rate of growth of lambs from five breeds of ewes that are commonly found in this section of the country. The results indicated that lambs from some of the breeds studied made a decided gain over others in the same period of time. Inasmuch as the sheep industry is of such economic importance to the Nation, the rate of growth of the lambs assumes more than ordinary interest, and an effort is being made to ascertain the relation of the quantity and composition of ewes' milk of each breed to the growth of the lambs.

FACTORS ESSENTIAL IN GROWTH

The chief factors in growth are inherited capacity and a sufficient quantity of nutritious food. The second factor only will be studied, since without sufficient food inherited capacity for growth will be more or less inhibited.

Growth depends upon nutritious foods, and recent investigators have demonstrated that these foods must contain a sufficient quantity of inorganic salts, certain amino acids, lipoids, fats or oils of a peculiar nature, and vitamines. The absence of any of these substances is detrimental to growth. It is obvious that a well-balanced food is essential, and in milk we have the highest type of such food. The fact, however, remains that milk from ewes of different breeds has been found to vary in quantity and composition, and this may account to some extent for differences in growth.

REVIEW OF LITERATURE

As early as 1850 data were collected giving the analysis of ewes' milk. Since that time many investigators[2] have contributed to our knowledge of the composition of ewes' milk. Their results, however, have dealt mainly with the high-milk-producing ewes of foreign countries, where

[1] The authors wish to acknowledge with thanks the careful work of the following men whose assistance made it possible to carry on this work: To Messrs. Grover D. Turnbow, R. R. Groninger, and Ronald Wood credit is due for the chemical analyses; to Messrs. O. W. Johnson, C. H. Ficke, and W. H. Booth (killed in service, France) for the careful determination on yield of milk and growth of lambs.

[2] KÖNIG, J. CHEMIE DER MENSCHLICHEN NAHRUNGS- UND GENUSSMITTEL. Aufl. 4, Bd. 1, p. 265–271. Berlin, 1903.

yield of milk and the butter-fat content was of greatest consequence. These results are of little benefit in the solution of this problem, other than to show the great variation that occurs between different breeds and within the breeds. This problem will include only such breeds as are common to this section of the country. Among the above-mentioned investigations, only two give figures upon breeds that will be included in this work. Filhol and Joly [1] give figures upon the Southdown, and Hucho [2] upon the Hampshire breed. These results serve only to verify the results of other investigators, and show the variation between breeds.

Fuller and Kleinheinz,[3] of the Wisconsin Station, made a study of the yield, fat, and total solids of the milk of five breeds of sheep; the Oxford, Southdown, Dorset, Shropshire, Merino, and the Montana grade. They included two ewes of each breed in their study, and took the average of the two results as the average of the breed. In determining the milk yield, the lamb was weighed before and after sucking the mother ewe. This was repeated at frequent intervals during a 48-hour period, from which the yield of milk for 24 hours was calculated. They observed that, when the ewes were milked by hand, only about one-half the quantity of milk was obtained as when the first method was used. The results on the two ewes of each breed show a wide variation in milk yield and percentage of fat between the breeds.

Ritzman,[4] of the New Hampshire Station, in a recent publication has made a valuable contribution to the present knowledge of ewes' milk. His work dealt especially with the fat content and its relation to growth of lambs. A summary of his results on the fat content of 6 distinct breeds and 11 crossbreeds over a considerable period of years showed a great variation in the percentage of fat. The outstanding feature was that not only did breeds differ in fat content of milk, but individual ewes within the breed differed greatly. Moreover, these individual ewes showed marked differences in fat percentage at different lactation periods. This fact was observed by the writers during a preliminary investigation of ewes' milk carried on a year previous to this present investigation. Ritzman concluded that the growth of the lamb was not dependent upon the percentage of fat, but he was of the opinion that it depended mainly on the quantity of milk. No actual milk yields were obtained by him, but an estimation of the yields made by observation was tabulated as "high-", "good-", "fair-", and "poor-milking" ewes.

From a review of the literature it is evident that an accurate estimation of the quantity and composition of ewes' milk is necessary in order

[1] FILHOL, and JOLY. ANALYSES DU LAIT DE BREBIS APPARTENANT À DIFFÉRENTES RACES. *In* Compt. Rend. Acad. Sci. [Paris] t. 47, no. 25, p. 1013–1014. 1858.

[2] HUCHO, Hermann. UNTERSUCHUNGEN ÜBER SCHAFMILCH MIT BBESONDERER BRÜCKSICHTIGUNG DER OSTFRIESISCHEN MILCHSCHAFE. *In* Landw. Jahrb., Bd. 26, Heft 2/3, p. 496–547. 1897.

[3] FULLER, J. G., and KLEINHEINZ, Frank. ON THE DAILY YIELD AND COMPOSITION OF MILK FROM EWES OF VARIOUS BREEDS. *In* Wis. Agr. Exp. Sta. 21st Ann. Rpt. 1903/04, p. 48–50. 1904.

[4] RITZMAN, E. G. EWES' MILK: ITS FAT CONTENT AND RELATION TO THE GROWTH OF LAMBS. *In* Jour. Agr. Research, v. 8, no. 2, p. 29–36, 1 fig. 1917. Literature cited, p. 35–36.

to ascertain the factors which influence growth, since analyses of the milk of individual ewes differ widely. In the first year's work, which was preliminary in nature, five breeds of ewes were studied, an estimation of the quantity of milk given by each ewe was made every seven days, on two ewes of each breed. Chemical analyses of the samples of milk taken in 10-day periods after lambing were made for a period of 70 days. The gain of the lamb was recorded every seven days. The chief objections showing up in the preliminary work were as follows: It became evident that samples of milk for analysis and total quantities of milk ought to be taken at the same period, or as near thereto as possible. The experiment included only two ewes in each breed, and in some cases one might give an abnormally high or low milk yield, which would show unfair averages in the breed. Still another factor entered into the work. A period of 70 days proved too long, for lambs need access to grain early in their life, and as grain was fed to them this made any correlation of composition of milk and growth futile. All the above difficulties were eliminated by the following procedure adopted in this work:

PLAN OF INVESTIGATION

It was realized that any work on the study of the milk of ewes must include a number of ewes before a fair average of the milk constituents could be obtained. However, in this work the difficulty becomes very evident, for with a great number of ewes the work becomes so laborious that the use of a great number in the experiment is prohibitive. The aim was to choose three ewes which showed characteristics of the average ewe of the particular breed. This was done by starting with four ewes of each breed and continuing with the three that showed the nearest to the normal milk yield for the breed. Six breeds of ewes and three ewes from each breed were used in this experiment. The period of investigation continued for 50 days. Every 10 days after lambing the total quantity of milk was recorded, and samples of milk were taken. The weight of the lamb was taken at birth and every 10 days thereafter, from which the gain was calculated.

METHODS USED IN OBTAINING MILK SAMPLES

In determining the total milk yield of each ewe the lamb was separated from the mother ewe at 6 o'clock in the morning. At 7 it was allowed to suckle the ewe. This was done in order to start all ewes on a uniform basis. At frequent intervals during the 24-hour period, which began after the lamb suckled the ewe at 7 o'clock, the lamb was weighed, allowed to suckle, and reweighed, on a balance weighing accurately to 1 gm. The sum of the differences in the lamb's weight before and after suckling the ewe during the 24-hour period gave the total yield of milk. In this manner all the milk was obtained without causing any nervousness on the part of the ewe, and the results gave a good

representative total yield of milk. The milk samples for the analysis were obtained as follows: After the 24-hour period was concluded for the total yield of milk the lamb was kept away from the ewe until a sufficient quantity of milk was in the udder; then the lamb was allowed to suckle one side, while the other was milked dry. In this manner a uniform sample was obtained without causing undue nervousness on the part of the ewe.

CONSTITUENTS DETERMINED IN THE MILK

The samples of milk were analyzed for the following constituents: Total nitrogen, casein, albumin, fat, lactose, specific gravity, and ash. The ash was then analyzed for the calcium and phosphorus content.

METHODS USED

TOTAL NITROGEN.—A quantity of milk (approximately 5 gm.) was weighed accurately and the nitrogen determined by the Kjeldahl method.

CASEIN.—Casein was precipitated by acetic acid on a weighed quantity of milk according to the official method. The nitrogen determined by the Kjeldahl method and the results multiplied by the factor 6.38.

ALBUMIN.—After neutralizing the filtrate obtained after removing the casein, with sodium hydroxid, and adding acetic acid of the proper strength and quantity, according to the official methods,[1] the nitrogen was determined by the Kjeldahl method and the result multiplied by 6.38.

NONPROTEIN NITROGEN.—The sum of the nitrogen of the casein and albumin was subtracted from the total nitrogen. The result gave the nonprotein nitrogen.

FAT.—The fat was determined by the Babcock method.

LACTOSE.—A portion of milk (approximately 10 gm.) was weighed accurately in a flask and 25 cc. of distilled water were added. The proteins were precipitated with a sufficient quantity of colloidal ferric hydroxid as described by Hill.[2] They were then filtered off and the clear filtrate collected in a volumetric flask. The proteins were washed with distilled water until free from lactose. The combined filtrate and washings were made up to a definite volume and the lactose determined by the volumetric method of Benedict.[3] The colloidal ferric hydroxid proved to be a very efficacious clarifier, as it is very simple to use and insures thorough clarification and a clear solution.

SPECIFIC GRAVITY.—Specific gravity was determined by the Westphal balance.

ASH.—The ash was made upon composite samples of the four samples of milk by the official methods.

CALCIUM AND PHOSPHORUS.—Calcium and phosphorus were determined from the ash residues by the methods described by Richmond.[4]

DISCUSSION OF RESULTS

In Table I is found the percentage composition and total yield of milk of each ewe for the entire series taken every 10 days during a period of 24 hours. In all cases the first results upon the total weight

[1] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. REPORT OF COMMITTEE ON EDITING METHODS OF ANALYSIS. p. 287–289. Baltimore, Md., 1916. (Jour. Assoc. Off. Agr. Chem., v. 2, no. 3, pt. 2.)

[2] HILL, REUBEN L. NOTE ON THE USE OF COLLOIDAL IRON IN THE DETERMINATION OF LACTOSE IN MILK. *In* Jour. Biol. Chem., v. 20, no. 3, p. 175–176. 1915.

[3] BENEDICT, STANLEY R. THE DETECTION AND ESTIMATION OF GLUCOSE IN URINE. *In* Jour. Amer. Med. Assoc., v. 57, no. 15, p. 1193–1194. 1911.

[4] RICHMOND, HENRY DROOP. DAIRY CHEMISTRY. p. 81–82. London. 1899.

of milk were secured 10 days after the birth of the lamb. This duration of time was allowed to elapse in order to allow the milk of the ewe to become normal. Analyses of ewes' milk, made by Weiske and Kennepohl[1] at different periods, varying from 1½ hours to several days after the birth of the lamb, show that 10 days is ample time for the milk flow to assume its normal composition.

The results of the table indicate, as would naturally be assumed, that there is a decrease in the milk flow of the ewes in the 50-day period. In only one instance was this not true; that was in the case of Cotswold ewe, No. 753, which maintained not only a constant milk flow throughout the experiment, but actually showed a slight increase at the end of the 50-day period.

TABLE I.—*Quantity and composition of ewes' milk*

Breed and No. of ewes.	Sample No.	Date of sampling.	Weight of ewe.	Total quantity of milk for 24-hour period.	Specific gravity.	Casein.	Albumin.	Nonprotein nitrogen.	Fat.	Lactose.	Ash.	Ash percentage of calcium and phosphorus in ash.	
												Calcium.	Phosphorus pentoxid.
COTSWOLD			*Lbs.*	*Gm.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
	1	Feb. 18	190	1,931	1.029	2.44	0.91	0.076	7.2	4.79	0.87	15.15	19.92
	2	Feb. 28	184	1,980	1.033	2.47	.88	.075	8.1	4.83	.....	.....	.....
2518	3	Mar. 10	189	1,805	1.031	3.60	.64	.087	8.6	4.00	.....	.....	.....
	4	Mar. 20	193	1,122	1.035	3.59	.78	.062	7.4	4.93	.....	.....	.....
	5	Mar. 30	188	1,176	1.033	3.52	.83	.067	5.2	5.12	.....	.....	.....
Average	....	.....	188	1,585	1.034	3.32	.81	.065	7.3	4.73	.87	15.15	19.92
	1	Feb. 21	164	1,956	1.033	2.86	.81	.070	10.4	4.81	.77	15.24	28.24
	2	Mar. 3	159	2,141	1.032	3.01	.78	.040	8.0	5.07	.....	.....	.....
753	3	Mar. 13	161	2,105	1.033	3.07	.45	.081	7.8	5.16	.....	.....	.....
	4	Mar. 23	157	1,637	1.033	3.10	.83	.051	6.4	4.79	.....	.....	.....
	5	Apr. 2	159	1,986	1.032	3.19	.54	.051	6.0	5.20	.....	.....	.....
Average	....	.....	159	1,965	1.033	3.04	.68	.058	7.7	5.00	.77	15.24	28.24
	1	Feb. 28	159	1,555	1.035	2.82	.72	.088	7.8	4.76	.34	11.33	18.77
	2	Mar. 10	150	1,302	1.028	3.04	.52	.087	9.2	4.93	.....	.....	.....
2097	3	Mar. 20	147	1,113	1.033	2.94	.64	.062	7.6	4.63	.....	.....	.....
	4	Mar. 30	145	838	1.035	3.04	.85	.067	7.8	4.60	.....	.....	.....
	5	Apr. 9	149	816	1.034	2.98	.88	.059	8.2	4.22	.....	.....	.....
Average	....	.....	149	1,124.8	1.033	2.96	.72	.072	8.1	4.62	.84	11.33	18.77
HAMPSHIRE													
	1	Feb. 17	189	2,477	1.029	2.84	.72	.081	10.3	4.58	.76	12.32	22.12
	2	Feb. 27	177	2,487	1.034	2.73	.81	.072	6.2	4.80	.....	.....	.....
30	3	Mar. 9	176	2,328	1.032	2.89	.48	.081	6.0	4.50	.....	.....	.....
	4	Mar. 19	165	1,845	1.035	3.62	.48	.062	7.6	4.88	.....	.....	.....
	5	Mar. 29	164	1,328	1.033	3.41	.48	.051	8.2	4.95	.....	.....	.....
Average	....	.....	174	2,093	1.032	3.09	.59	.069	7.6	4.74	.76	12.32	22.12
	1	Feb. 20	172	3,439	1.030	2.62	.91	.078	8.25	4.61	.81	14.22	24.65
	2	Mar. 2	152	2,278	1.031	3.77	.43	.059	6.85	4.53	.....	.....	.....
50	3	Mar. 12	151	2,534	1.030	2.84	.36	.054	6.2	4.86	.....	.....	.....
	4	Mar. 22	136	2,300	1.031	3.36	1.36	.056	5.0	4.97	.....	.....	.....
	5	Apr. 1	139	1,848	1.035	3.07	.52	.059	3.9	4.73	.....	.....	.....
Average	....	.....	150	2,479.8	1.031	3.13	.71	.061	6.0	4.74	.81	14.22	24.65

[1] WEISKE, H., AND KENNEPOHL, G. UNTERSUCHUNGEN ÜBER SCHAFMILCH UNTER VERSCHIEDENEN VERHÄLTNISSEN. *In* Jour. Landw., Jahrg. 29, p. 451–472. 1881.

TABLE I.—*Quantity and composition of ewes' milk*—Continued

Breed and No. of ewes.	Sample No.	Date of sampling.	Weight of ewe.	Total quantity of milk for 24-hour period.	Specific gravity.	Casein.	Albumin.	Nonprotein nitrogen.	Fat.	Lactose.	Ash.	Ash percentage of calcium and phosphorus in ash.	
												Calcium.	Phosphorus pentoxid.
HAMPSHIRE—con.			*Lbs.*	*Gm.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
	1	Feb. 23	189	3,103	1.030	2.83	1.27	0.11	11.2	4.59	0.78	15.19	27.68
	2	Mar. 5	169	2,159	1.033	3.04	1.11	.09	6.0	4.83	.....	.....	.....
33	3	Mar. 15	176	1,352	1.030	3.33	.59	.06	6.8	4.93	.....	.....	.....
	4	Mar. 25	168	1,505	1.032	2.88	.81	.09	6.8	4.86	.....	.....	.....
	5	Apr. 4	169	1,573	1.033	2.81	.82	.08	7.4	4.42	.....	.....	.....
Average	....	.....	176	1,938.4	1.032	2.97	.92	.08	7.6	4.72	.78	15.19	27.68
SOUTHDOWN													
	1	Feb. 23	129	1,747	1.031	3.11	1.14	.094	11.35	4.94	.76	15.39	23.78
	2	Mar. 8	127	1,383	1.027	3.24	1.04	.070	8.0	4.98	.....	.....	.....
89	3	Mar. 15	128	988	1.034	4.03	.62	.067	5.6	4.97	.....	.....	.....
	4	Mar. 25	124	863	1.030	3.18	.86	.090	8.8	4.98	.....	.....	.....
	5	Apr. 4	126	753	1.034	3.60	.66	.030	7.4	4.73	.....	.....	.....
Average	....	.....	127	1,146.8	1.031	3.43	.86	.070	8.2	4.92	.76	15.39	23.78
	1	Feb. 26	110	1,754	1.040	2.51	.80	.064	5.2	5.02	.78	14.43	28.68
	2	Mar. 5	111	1,521	1.030	3.52	.88	.044	5.3	4.86	.....	.....	.....
128	3	Mar. 18	111	1,368	1.033	3.73	.48	.064	8.0	4.97	.....	.....	.....
	4	Mar. 28	108	1,393	1.035	2.54	.67	.056	5.6	4.96	.....	.....	.....
	5	Apr. 7	104	1,317	1.034	4.01	.64	.078	7.0	4.59	.....	.....	.....
Average	....	.....	109	1,470.6	1.034	3.26	.69	.060	6.2	4.88	.78	14.43	28.68
	1	Mar. 10	136	1,551	1.035	3.25	.83	.120	7.6	4.01	1.19	12.22	20.96
	2	Mar. 20	133	1,262	1.035	3.72	.84	.070	8.0	4.72	.....	.....	.....
207	3	Mar. 30	132	1,051	1.035	3.84	.77	.070	9.0	4.82	.....	.....	.....
	4	Apr. 9	134	895	1.037	3.76	.74	.030	7.9	3.94	.....	.....	.....
	5	Apr. 19	130	743	1.035	3.74	.91	.050	7.9	4.07	.....	.....	.....
Average	....	.....	133	1,100.4	1.035	3.66	.82	.068	8.0	4.31	1.19	12.22	20.96
SHROPSHIRE													
	1	Mar. 12	153	1,417	1.035	3.97	.65	.07	8.0	4.73	.95	17.03	27.31
	2	Mar. 22	156	1,596	1.035	2.83	.48	.08	8.4	4.83	.....	.....	.....
752	3	Apr. 1	147	1,468	1.035	3.64	.65	.07	7.6	4.97	.....	.....	.....
	4	Apr. 11	142	1,037	1.035	3.66	.47	.06	7.8	4.07	.....	.....	.....
	5	Apr. 21	146	1,018	1.035	3.77	.56	.02	8.8	3.84	.....	.....	.....
Average	....	.....	149	1,307.2	1.035	3.57	.56	.06	8.1	4.48	.95	17.03	27.31
	1	Mar. 15	167	2,602	1.034	3.98	.67	.09	6.4	4.88	.84	21,78	34.73
	2	Mar. 25	156	2,144	1.033	3.97	.72	.07	8.0	4.89	.....	.....	.....
366346	3	Apr. 4	156	2,148	1.034	3.95	.78	.09	6.4	4.73	.....	.....	.....
	4	Apr. 14	151	1,836	1.033	3.36	.88	.09	7.2	4.12	.....	.....	.....
	5	Apr. 24	150	1,524	1.039	3.34	.89	.07	8.0	3.82	.....	.....	.....
Average	....	.....	156	2,050.8	1.034	3.72	.79	.08	7.2	4.48	.84	21.78	34.73
	1	Mar. 14	159	1,499	1.030	3.37	.67	.066	7.2	5.10	.87	19.04	30.57
	2	Mar. 24	156	1,701	1.037	3.06	.88	.042	10.4	4.90	.....	.....	.....
49	3	Apr. 3	153	1,088	1.030	3.11	.89	.092	9.6	4.73	.....	.....	.....
	4	Apr. 13	149	996	1.032	3.08	.92	.09	8.8	4.22	.....	.....	.....
	5	Apr. 23	149	924	1.034	2.98	.96	.09	9.6	3.91	.....	.....	.....
Average	....	.....	153	1,241.8	1.032	3.12	.86	.076	9.1	4.57	.87	19.04	30.57
LINCOLN													
	1	Feb. 21	192	1,528	1.013	2.98	.81	.06	10.8	4.72	.82	16.95	28.62
	2	Mar. 3	191	1,456	1.036	2.88	.89	.043	9.0	4.71	.....	.....	.....
1940	3	Mar. 13	191	1,193	1.027	3.28	.56	.076	7.2	4.89	.....	.....	.....
	4	Mar. 23	186	1,191	1.025	2.98	.83	.087	8.0	4.82	.....	.....	.....
	5	Apr. 2	188	1,180	1.026	2.83	.52	.073	8.8	4.73	.....	.....	.....
Average	....	.....	189	1,309.6	1.023	2.97	.72	.067	8.8	4.77	.82	16.95	28.62

TABLE I.—*Quantity and composition of ewes' milk*—Continued

Breed and No. of ewes.	Sample No.	Date of sampling.	Weight of ewe.	Total quantity of milk for 24-hour period.	Specific gravity.	Casein.	Albumin.	Nonprotein nitrogen.	Fat.	Lactose.	Ash.	Ash percentage of calcium and phosphorus in ash,	
												Calcium.	Phosphorus pentoxid.
LINCOLN—contd.			*Lbs.*	*Gm.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1913	1	Feb. 27	187	1,955	1.033	2.92	1.12	.101	7.6	4.74	0.67	15.20	27.56
	2	Mar. 9	179	1,569	1.033	3.22	.84	.062	6.0	4.72	.....	.....	.....
	3	Mar. 19	176	1,441	1.033	2.98	.58	.059	6.9	4.87	.....	.....	.....
	4	Mar. 29	176	1,274	1.035	3.39	.95	.077	6.4	4.60	.....	.....	.....
	5	Apr. 8	176	1,482	1.035	3.38	.91	.078	7.4	4.70	.....	.....	.....
Average	....	.....	179	1,544.2	1.033	3.17	.88	.075	6.8	4.72	.67	15.20	27.56
1996	1	Feb. 24	193	1,574	1.032	2.60	1.19	.100	9.6	4.75	.80	14.73	29.13
	2	Mar. 6	180	1,313	1.032	2.67	1.26	.084	7.2	4.85	.....	.....	.....
	3	Mar. 16	182	735	1.029	3.66	.45	.037	11.4	4.93	.....	.....	.....
	4	Mar. 26	185	481	1.030	3.25	.40	.011	8.2	5.12	.....	.....	.....
	5	Apr. 5	175	506	1.032	3.35	.39	.032	8.2	4.30	.....	.....	.....
Average	....	.....	183	921.8	1.031	3.10	.73	.053	8.9	4.79	.80	14.73	29.13
RAMBOUILLET													
36	1	Feb. 12	136	1,918	1.033	3.63	.86	.062	10.05	4.82	.91	18.49	29.38
	2	Feb. 22	131	1,391	1.037	3.62	.75	.029	11.9	4.78	.....	.....	.....
	3	Mar. 4	130	1,347	1.032	4.75	.52	.048	9.6	5.00	.....	.....	.....
	4	Mar. 14	130	1,131	1.034	4.22	1.23	.037	9.6	4.60	.....	.....	.....
	5	Mar. 24	131	1,112	1.033	4.58	.84	.070	8.2	4.68	.....	.....	.....
Average	....	.....	132	1,379.8	1.034	4.16	.84	.059	9.8	4.77	.91	18.49	29.38
59	1	Feb. 26	156	2,582	1.039	3.42	.98	.110	7.4	5.00	.80	16.64	31.08
	2	Mar. 8	151	2,113	1.032	3.05	.95	.019	6.8	4.97	.....	.....	.....
	3	Mar. 18	149	1,766	1.040	3.40	.48	.064	6.4	5.08	.....	.....	.....
	4	Mar. 28	147	1,706	1.034	3.17	.74	.062	5.9	5.07	.....	.....	.....
	5	Apr. 7	144	1,758	1.033	3.59	.51	.029	7.0	4.56	.....	.....	.....
Average	....	.....	149	1,985	1.035	3.32	.73	.056	6.7	4.93	.80	16.64	31.08
74	1	Feb. 28	169	1,525	1.042	3.64	.94	.067	3.4	4.71	.86	19.64	32.47
	2	Mar. 10	167	1,262	1.030	3.45	1.27	.054	9.6	4.76	.....	.....	.....
	3	Mar. 20	160	1,140	1.034	3.21	.72	.091	6.4	4.79	.....	.....	.....
	4	Mar. 30	164	880	1.037	2.96	.58	.057	8.0	4.75	.....	.....	.....
	5	Apr. 9	165	802	1.034	3.45	.86	.062	8.2	4.00	.....	.....	.....
Average	....	.....	165	1,121.8	1.035	3.34	.87	.066	7.1	4.60	.86	19.64	32.47

A study of Table I brings out the fact that there is a great variability in the percentages of the constituents of ewes' milk. Not only is this true among the different breeds, but also during the lactation period of the individual. The most constant constituent in the milk of all breeds examined appears to be lactose, while fat seems to be the most variable. The difference in the percentages of fat is very marked, not only between the breeds, but during the lactation period of the individual. These observations are in harmony with the results secured by Ritzman,[1] who also found that the fat varied at different lactation periods of individual ewes and who concluded that—

No great reliance can be placed on single tests of an individual, and that a test must either cover a larger number of periods during one lactation of an individual or that

[1] RITZMAN, E. G., 1917. OP. CIT., p. 31.

it must cover an average of a large number of individuals at one period, in order to be representative.

When the average percentages of fat for the five lactation periods of each ewe are determined and compared, the variation of fat content is not so marked, which indicates clearly the value of a number of tests rather than one single test on an individual.

TABLE II.—*Average quantity and composition of milk for each ewe and for each breed*

Breed and No. of ewe.	Average total quantity of milk for 24-hour periods.	Specific gravity.	Composition of milk.					
			Casein.	Albumin.	Non-protein.	Fat.	Lactose.	Ash.
COTSWOLD	*Gm.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
2518	1,585	1.034	3.32	0.81	0.065	7.3	4.73	0.87
753	1,965	1.032	3.04	.68	.058	7.7	5.00	.77
2097	1,124.8	1.033	2.96	.72	.072	8.1	4.62	.84
Average	1,558	1.033	3.10	.74	.065	7.7	4.78	.82
HAMPSHIRE								
50	2,479	1.031	3.13	.71	.061	6.0	4.74	.81
33	1,938.4	1.032	2.97	.92	.080	7.6	4.72	.78
30	2,093	1.032	3.09	.59	.069	7.6	4.74	.76
Average	2,170	1.032	3.06	.74	.070	7.1	4.73	.78
LINCOLN								
1940	1,309.6	1.023	2.97	.72	.067	8.8	4.77	.82
1996	921.8	1.033	3.17	.88	.077	6.8	4.72	.67
1913	1,544.2	1.025	3.10	.73	.053	8.9	4.79	.80
Average	1,258	1.027	3.08	.77	.065	8.1	4.76	.76
RAMBOUILLET								
36	1,379.8	1.039	4.16	.84	.059	9.8	4.77	.91
59	1,985	1.035	3.32	.73	.056	6.7	4.93	.80
74	1,121.8	1.035	3.34	.87	.066	7.1	4.60	.86
Average	1,495	1.036	3.60	.81	.078	7.8	4.77	.85
SOUTHDOWN								
89	1,146.8	1.031	3.43	.86	.070	8.2	4.92	.76
128	1,470.6	1.034	3.26	.69	.060	6.2	4.88	.78
207	1,100.4	1.035	3.66	.82	.068	8.0	4.31	1.19
Average	1,238	1.033	3.45	.79	.066	7.5	4.70	.91
SHROPSHIRE								
752	1,307.2	1.035	3.57	.56	.06	8.1	4.48	.95
366346	2,050.8	1.034	3.72	.79	.08	7.2	4.48	.84
49	1,241.8	1.032	3.12	.86	.07	9.1	4.57	.87
Average	1,532	1.033	3.47	.77	.07	8.1	4.50	.88

Tables II and III are given for convenience of comparison of the average yield and the average analysis of the milk for the 50-day period. Table II gives the averages for the three individual ewes of each breed, and the average of these averages is represented in Table III as the average for the breed.

TABLE III.—*Average quantity and composition of milk for each breed*

Name of breed.	Average total quantity of milk for 24-hour periods.	Specific gravity.	Composition of milk.					
			Casein.	Albumin.	Non-protein.	Fat.	Lactose.	Ash.
	Gm.		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Hampshire	2,170	1.032	3.06	0.74	0.070	7.1	4.72	0.78
Cotswold	1,558	1.033	3.10	.74	.065	7.7	4.45	.82
Shropshire	1,532	1.033	3.47	.77	.007	8.1	4.50	.88
Rambouillet	1,495	1.036	3.60	.81	.078	7.8	4.77	.85
Lincoln	1,258	1.027	3.08	.77	.065	8.1	4.76	.76
Southdown	1,238	1.033	3.45	.79	.066	7.5	4.70	.91

Table III brings out clearly the differences in milk yields for the different breeds. The Hampshire ewes in this experiment easily ranked first in quantity of milk produced, while the differences in the other five breeds were not so great.

TABLE IV.—*Initial weight of lambs and their gain during each 10-day period*

Breed and No. of ewe.	Number of lambs.	Initial weight of lambs.		Amount of weight gained by lambs each 10-day period.		
		First.	Second.	First.	Second.	Total.
SHROPSHIRE		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
752	1	4,294	.....	2,392	.....	2,392
				2,468	.....	2,468
				2,902	.....	2,902
				2,032	.....	2,032
				1,957	.....	1,957
Total gain 50 days	.....	.....	.....	11,751	.....	11,751
366346	2	3,955	4,407	2,535	2,518	5,053
				2,110	2,304	4,414
				1,372	1,452	2,824
				1,644	1,637	3,281
				1,522	1,374	2,896
Total gain 50 days	.....	.....	.....	.....	.....	18,468
49	2	3,503	3,277	1,682	1,888	3,570
				1,139	1,085	2,224
				1,395	1,023	2,418
				1,360	977	2,337
				751	761	1,511
Total gain 50 days	.....	.....	.....	.....	.....	12,060
LINCOLN						
1940	1	5,311	.....	2,507	.....	2,507
				2,269	.....	2,269
				2,296	.....	2,296
				2,089	.....	2,089
				2,111	.....	2,111
Total gain 50 days	.....	.....	.....	11,272	.....	11,272

TABLE IV.—*Initial weight of lambs and their gain during each 10-day period*—Contd.

Breed and No. of ewe.	Number of lambs.	Initial weight of lambs.		Amount of weight gained by lambs each 10-day period.		
		First.	Second.	First.	Second.	Total.
LINCOLN—continued		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1913	1	5,650	.....	3,003	.....	3,003
				2,754	.....	2,754
				2,256	.....	2,256
				2,286	.....	2,286
				1,782	.....	1,782
Total gain 50 days	.....	.....	.....	12,081	.....	12,081
1996	1	5,424	.....	2,463	.....	2,463
				2,078	.....	2,078
				[a] 844	.....	[a] 844
				696	.....	696
				702	.....	702
Total gain 50 days	.....	.....	.....	6,783	.....	6,783
RAMBOUILLET						
36	1	5,085	.....	2,189	.....	2,189
				3,114	.....	3,114
				2,117	.....	2,117
				2,395	.....	2,395
				1,681	.....	1,681
Total gain 50 days	.....	.....	.....	11,496	.....	11,496
59	2	4,181	4,181	2,526	2,230	4,756
				249	1,969	2,218
				1,140	1,722	2,862
				770	1,681	2,451
				1,612	1,423	3,035
Total gain 50 days	.....	.....	.....	.....	.....	16,092
74	2	3,616	3,390	1,427	937	2,364
				542	598	1,140
				992	780	1,772
				755	795	1,550
				1,012	1,227	2,239
Total gain 50 days	.....	.....	.....	.....	.....	9,065
COTSWOLD						
2518	2	4,633	4,520	2,431	2,318	4,749
				1,527	1,587	3,114
				1,305	1,566	2,871
				1,260	1,363	2,623
				1,222	1,152	2,374
Total gain 50 days	.....	.....	.....	.....	.....	15,751
753	1	4,068	.....	3,696	.....	3,696
				3,549	.....	3,549
				2,976	.....	2,976
				2,995	.....	2,995
				2,191	.....	2,191
Total gain 50 days	.....	.....	.....	15,407	.....	15,407

[a] Lamb sick, did not thrive.

TABLE IV.—*Initial weight of lambs and their gain during each 10-day period*—Contd.

Breed and No. of ewe.	Number of lambs.	Initial weight of lambs.		Amount of weight gained by lambs each 10-day period.		
		First.	Second.	First.	Second.	Total.
COTSWOLD—continued		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
2097	1	5,650	.....	2,945	.....	2,945
				1,861	.....	1,861
				1,561	.....	1,561
				1,646	.....	1,646
				1,796	.....	1,796
Total gain 50 days	.....	.....	.....	9,809	.....	9,809
HAMPSHIRE						
30	2	5,424	3,503	1,932	1,679	3,611
				2,919	2,479	5,398
				1,937	1,149	3,086
				2,308	1,527	3,835
				1,934	822	2,756
Total gain 50 days	.....	.....	.....	.....	.....	18,686
50	2	4,407	4,859	3,735	3,338	7,073
				2,213	2,202	4,415
				1,598	2,073	3,681
				2,061	2,275	4,336
				1,416	2,315	3,731
Total gain 50 days	.....	.....	.....	.....	.....	23,236
33	1	4,294	.....	6,349	.....	6,349
				3,814	.....	3,814
				2,769	.....	2,769
				2,835	.....	2,835
				2,317	.....	2,317
Total gain 50 days	.....	.....	.....	18,084	.....	18,084
SOUTHDOWN						
89	1	4,294	.....	2,764	.....	2,764
				2,752	.....	2,752
				2,239	.....	2,239
				1,864	.....	1,864
				982	.....	982
Total gain 50 days	.....	.....	.....	10,601	.....	10,601
128	1	4,068	.....	2,995	.....	2,995
				2,443	.....	2,443
				2,592	.....	2,592
				1,950	.....	1,950
				1,789	.....	1,789
Total gain 50 days	.....	.....	.....	11,769	.....	11,769
207	2	3,616	3,503	1,503	1,555	3,058
				983	1,280	2,263
				815	1,207	2,022
				886	1,524	2,410
				1,247	1,483	2,730
Total gain 50 days	.....	.....	.....	.....	.....	12,483

In Table IV data are given on the initial weight of the lamb or lambs and the gain in weight every 10 days during the period of the experiment. The total gain is also included.

Table V is a combination of the results on total milk yield and the total constituents of the milk, expressed in grams, calculated from the average percentages secured on the 50-day period, and also data on the total gain in weight of the lambs.

Table VI gives the averages of the above constituents for each breed.

TABLE V.—*Relation of Milk Constituents of Individual Ewes to Growth of Lambs*

Breed and No. of ewe.	Total quantity of milk.	Total casein.	Total albumin.	Total non-proteids.	Total fat.	Total lactose	Total ash.	Weight at birth.		Number of lambs to ewe.	Total growth.
								Lamb No. 1.	Lamb No. 2.		
HAMPSHIRE	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*Gm.*
30	104,650	3,237	617	72	7,953	4,960	795	5,424	3,505	[a] 2	21,764
50	123,950	3,897	880	76	7,437	5,974	1,004	4,407	4,859	2	23,236
33	96,920	2,878	891	77	7,375	4,574	756	4,294	.....	1	18,084
Average	108,506.6	3,331	796	75	7,585	5,126	852	.....	.....	.....	21,028
COTSWOLD											
753	98,250	2,987	668	57	7,565	4,912	756	4,068	.....	1	15,407
2518	79,250	2,631	642	52	5,785	3,748	689	4,633	4,520	2	17,731
2097	56,240	1,664	405	40	4,555	2,588	472	5,650	.....	1	9,809
Average	77,913.3	2,427	572	50	5,968	3,749	639	.....	.....	.....	14,318
RAMBOUILLET											
36	68,990	2,870	579	41	6,761	3,280	627	5,085	3,842	[b] 1	13,078
59	99,250	3,295	705	56	6,650	4,913	794	4,181	4,181	2	15,322
74	56,090	1,873	487	37	3,982	2,680	482	3,616	3,390	2	9,065
Average	75,110	2,677	590	45	5,797	3,624	634	.....	.....	.....	12,488
LINCOLN											
1940	65,450	1,944	471	44	5,650	3,122	537	5,311	.....	1	11,272
1913	77,210	2,393	564	41	6,871	3,698	618	5,424	.....	1	12,081
1996	46,090	1,461	406	35	3,135	2,176	309	5,650	.....	1	6,783
Average	63,250	1,933	480	40	5,219	2,999	388	.....	.....	.....	10,045
SOUTHDOWN											
89	57,340	1,967	493	40	4,702	2,821	435	4,294	.....	1	10,601
128	73,530	2,397	507	51	5,559	3,588	573	4,068	.....	1	11,769
207	55,020	2,013	451	37	4,401	2,371	654	3,616	3,503	2	12,483
Average	61,963.3	2,136	484	43	4,554	2,926	554	.....	.....	.....	11,618
SHROPSHIRE											
753	65,360	2,333	366	39	5,294	2,928	621	4,294	.....	1	11,751
366346	102,540	3,814	810	82	7,382	4,594	861	3,955	4,407	2	18,468
49	62,090	1,937	534	43	5,650	2,837	540	3,503	3,277	2	12,060
Average	76,663.3	2,695	570	55	6,109	3,452	675	.....	.....	.....	14,093

[a] Ewe had triplets, one was taken away on ninth day.
[b] Lamb had leg broken on ninth day and was removed.

In a comparison of the total quantity of milk constituents and the total growth of the lambs there is one disturbing factor. In all breeds, with the exception of the Lincoln, twins were born to one or more ewes in each breed, and in one case triplets. The three Lincoln ewes all gave birth to single lambs. It is obvious that in comparison of quantity

of milk and growth of lambs the best experimental results in this investigation would have been obtained if all ewes were allowed to raise only one lamb. In future work it is hoped that this condition may be fulfilled. However, many factors prevented such an arrangement. At the time of this investigation, which is an outgrowth of a more extended investigation on sheep, it was desired to make the work correspond as closely as possible to the actual conditions found in sheep husbandry, and other data were collected besides those included in this paper.

TABLE VI.—*Relation of Milk Constituents of Breeds to Growth of Lambs*

Breed.	Total quantity of milk.	Total casein	Total albumin.	Total nonproteids.	Total fat.	Total lactose.	Total ash.	Number of lambs to ewes.	Total growth.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*Gm.*
Hampshire.[1]	108,506.6	3,331	796	75	7,585	5,126	852	5	21,028
Cotswold	77,913.3	2,427	572	50	5,968	3,749	639	4	14,318
Shropshire	76,636.3	2,695	570	55	6,107	3,452	674	5	14,093
Rambouillet	75,110	2,677	590	45	5,797	3,624	634	5	12,488
Lincoln	63,250	1,933	480	40	5,219	2,999	388	3	10,045
Southdown	61,963.3	2,136	484	43	4,554	2,926	554	4	11,618

It is quite evident that twin lambs, given a sufficient quantity of milk, will make a greater total gain than a single lamb, provided their initial weights correspond and they are equally strong at birth. A certain amount of milk is essential for the growth of a lamb, but on the other hand there is a limit to the amount of milk that an animal can assimilate. Therefore, two lambs, given a sufficient quantity of milk, will have an advantage in total gain over a single lamb. The single lamb, however, is generally larger than either of the twin lambs at birth, but from an economical standpoint it is obvious that twins are more desirable in the flock than singles.

A compilation of the data on the Hampshire breed shows the single lamb of ewe No. 33 gained nearly as much in the same period of time as the twin lambs of ewe No. 30. A comparison of the total yield of milk shows ewe No. 33 produced slightly less than ewe No. 30.

In the Cotswold breed we have ewe No. 753 giving more than either of the other two ewes, and the single lamb has made a gain almost equal to the gain of the twin lambs of ewe No. 2518. Ewe No. 2518 has twin lambs, and their total gain is only slightly greater than the single lamb of ewe No. 753. The third ewe, No. 2097, shows a smaller milk yield than ewe No. 2518 with the twin lambs, and the gain of her single lamb is a little more than one-half as much as the total gain of the twin lambs. In the Rambouillet and Southdown breeds we find the total gain in weight of the lambs is proportional to the amount of milk consumed. In the Lincoln breed, the only breed where there are three single lambs, their gain in weight is also proportional to the quantity of milk consumed. However, the lamb belonging to the Lincoln ewe, No. 1996, became sick at the end of 20 days and did not thrive thereafter.

It appears from this experiment that the greatest factor in growth is quantity of milk; hence, a high-milk-producing ewe is more valuable than a low one. The inherited capacity for growth, however, must not be overlooked. As to the relative merits of the breeds, it is not the purpose of this investigation to enter upon a discussion. To draw conclusions upon such a small number of ewes in each breed would be unfair. It was the aim of this experiment to make the investigation as fair as possible to all breeds studied, and the authors desire to emphasize clearly the fact that results upon the different breeds are given wholly as an attempt to correlate milk yields, their composition, and their relation to growth. The results are not given with an idea of comparing the desirability or undesirability of the breeds included in this experiment, but rather for the purpose of presenting to the farmer and student information in regard to features of certain well-known breeds that have to do with utility and adaptation to certain specific purposes. For example, the man interested in the growth of lambs for early marketing would be interested in a breed that by its yield of milk, and possibly certain other factors, made the greatest average growth of lambs. Another purpose of the experiment is to stimulate the interest of investigators and students of animal breeding in the field for the improvement of certain breeds with reference to factors having to do with profit for the grower. There might even be room for a new breed that would possess all the desirable and highly useful factors of some of the breeds included in this experiment.

SEED DISINFECTION BY FORMALDEHYDE VAPOR

[PRELIMINARY REPORT]

By CECIL C. THOMAS

Pathological Inspector, Federal Horticultural Board, United States Department of Agriculture

INTRODUCTION

The continual introduction of plants by the Department of Agriculture, chiefly by means of seeds, from all parts of the world, and the constant danger of allowing little-known or serious diseases to enter thereon, emphasize the necessity for a study of the methods of seed disinfection. There are few data on this subject except in the case of cereals and for a small number of seeds used in physiological experiments.

The pathological inspectors of the Federal Horticultural Board have encountered many difficulties in treating hundreds of lots of seeds of widely varying types and quantities with the various liquid treatments in common use. Most of the treatments recommended and used at the present time require dipping or soaking in a water solution of some fungicide or germicide. The seeds, therefore, remain wet for a longer or shorter period, depending on the treatment given and the method of drying.

Some seeds like wheat and rye absorb water slowly and can be dried without much injury, while seeds like the various members of the mustard family absorb water very readily and with even a very brief treatment swell sufficiently to break the seed coat and allow the cotyledons to fall apart, thus destroying the seed.

Light seeds such as are found in many of the grasses present another problem for the wet treatment. It is very difficult to give them anything like a uniform treatment because of the difficulty of wetting them and keeping them under the liquid. Seeds such as flax, which have a mucilaginous covering, present still another difficulty for wet treatments.

The large number of shipments and the great variety of seeds passing through the quarantine inspection house of the Federal Horticultural Board, United States Department of Agriculture, that need to be treated render desirable the adoption of a method of treatment which will obviate wetting and drying. An attempt, therefore, is being made to develop a treatment of this type with formaldehyde vapor. While it is far from being perfected, it seems desirable to make a preliminary report on some of the results obtained.

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The pathological inspectors of the Federal Horticultural Board have treated several hundred lots of seeds each year in the liquid treatments commonly recommended and have found the formaldehyde solutions the best for the greatest number of cases. Formaldehyde is also known to be a very efficient germicide when used in the form of a vapor as a disinfectant for contagious human diseases.

For the above reasons formaldehyde vapor has been selected for this work.

APPARATUS

A galvanized iron can (fig. 1, A) having a capacity of approximately 130,000 cc. was used. After introducing the seeds and organisms,

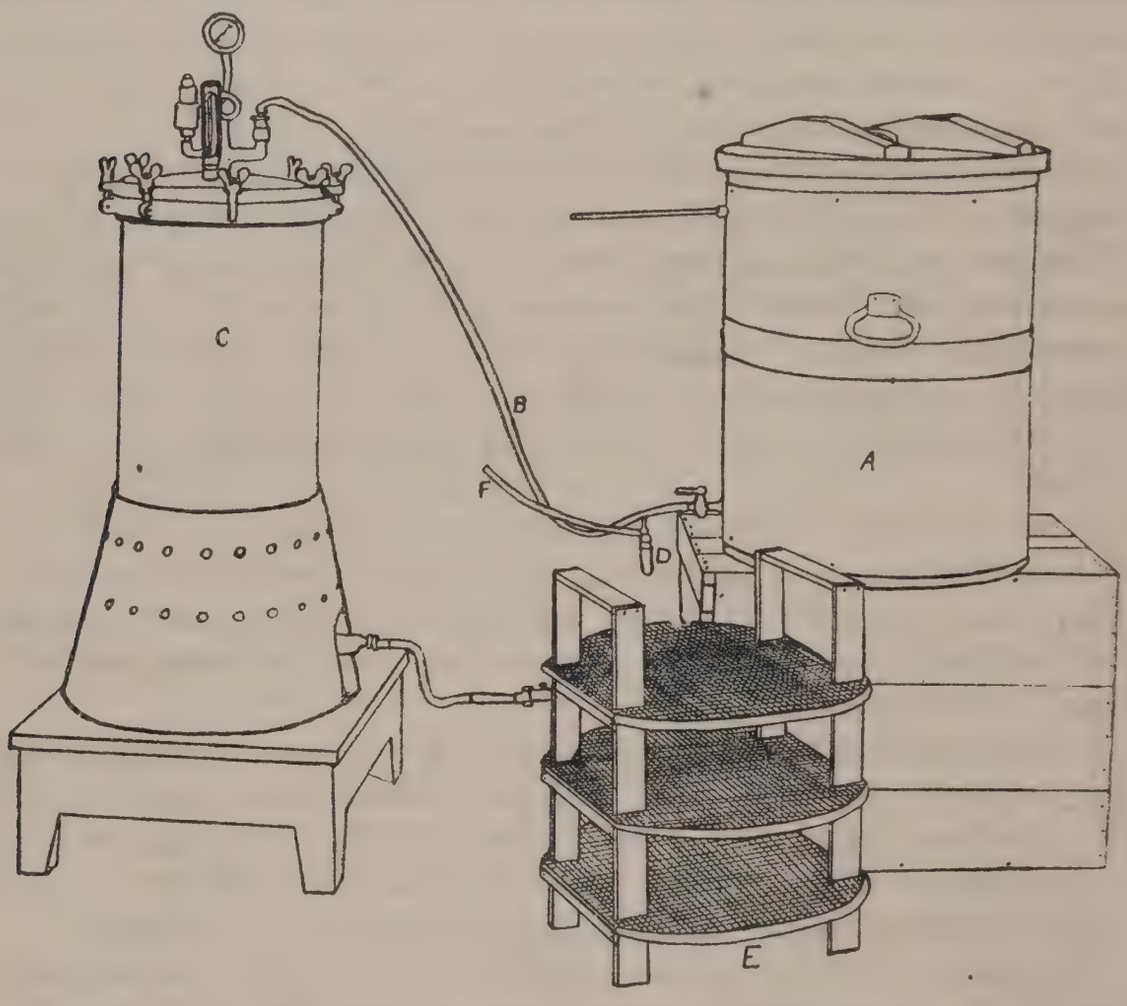

Fig. 1—Formaldehyde-vapor disinfecting apparatus

steam was added through a rubber tube (B) from an autoclave (C). The formaldehyde solution (Shoemaker and Busch, U. S. P. VIII, 40 per cent by volume of formic aldehyde) was diluted one to one with water to give a greater volume of liquid. This formaldehyde solution was introduced through an atomizer (D) with the aid of compressed air (F), the nozzle of the atomizer being inserted in the rubber tube (B), through which the steam passes as it enters the can. This insertion was made as close to the can as possible. Steam was first introduced and then the compressed-air tube was attached to the atomizer and the formaldehyde solution was forced in while the steam was still entering.

The finely atomized formaldehyde solution thus enters the can and is carried to all parts of it with the steam. Condensation takes place on the surface of the seeds, forming a thin film of moisture about each seed in which the formaldehyde may act, and as this film evaporates the gas is freed.

A frame (E) containing three wire shelves was used inside the can, and the seeds were placed in porcelain dishes on these shelves.

EXPERIMENTS

There are two distinct phases of this problem: (1) The effect on the seeds and (2), the effect on the fungi and bacteria.

Table I gives the result of a series of treatments of a number of different seeds. The formaldehyde solution, before dilution, was used at the rate of 10 ounces per 1,000 cubic feet to procure the results given in the second, third, and fourth columns and for the time indicated. The results given in the sixth column are for formaldehyde used at the rate of 30 ounces per 1,000 cubic feet for 2 hours.

The germination percentages given in this table are an average of the results obtained by the Seed Laboratories, Bureau of Plant Industry, United States Department of Agriculture, from germinating two samples of 100 seeds in each case.

TABLE I.—*Effect of formaldehyde vapor of different strengths for varying lengths of time on the germination of seed*

Seed.	Checks.	Strength formalin.				
		10 ounces per 1,000 cubic feet.			Checks.	30 ounces per 1,000 cubic feet (2 hours).
		1 hour.	2 hours.	3 hours.		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Alfalfa (C. I. 44)[1]	95.5	92	93.5	93.5	96	89.5
Barley (C. I. 25)[1]	92.5	94.5	93.5	94	90.5	92.5
Beet	71.5	58.5	64	66	66	68.5
Carrot, Oxheart	69.5	72.5	66.5	83.5	72.5	79
Clover, Crimson	70.5	69	75.5	65	69	64
Corn, Miner's Yellow Dent	96.5	95.5	96.5	97	97	98
Field Pea	93.5	94.5	89.5	93	93.5	89
Flax	94.5	92	93.5	93	86	85
Lettuce	97.5	97	98	99	99	99
Millet	94.5	92.5	93	92.5	81.5	88.5
Muskmelon, Rocky Ford	87	82.5	88	95.5	95	93.5
Natal Grass	1.5	3.5	3	4.5	3	0
Oats (C. I. 541–4)[1]	98	98	99	97	98	98
Orchard Grass	64.5	63.5	72.5	73.5	76.5	61.5
Radish, Icicle	97	98.5	97	98	96	96.5
Rice (C. I. 1561)[1]	93.5	94	96	92	93.5	95
Rye (C. I. 138)[1]	83.5	84.5	89.5	87	83	86
Soy Bean	95.5	96	96.5	98	99	97.5
Sudan Grass	84	90.5	87.5	84	80	84.5
Wheat, Blue Stem (C. I. 1912–11)[1]	57.5	61.5	59.5	55.5	62	61.5

[1] These are accession numbers of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, from which some of the seeds were obtained.

Apparently there was little or no injury in any case. As shown by the percentage of germination in the checks the killing of all seeds in the case of Natal grass where 30 ounces per thousand cubic feet for 2 hours was used probably was due to the low vitality of the seeds.

A number of experiments have been conducted with fungi and bacteria in which they were treated with various amounts of the formaldehyde vapor and for different lengths of time. Five different organisms, *Monilia fructigena*, *Colletotrichum glocosporioides*, *Fusarium vasinfectum*, *Ascochyta* sp., and *Bacillus caratovorus* were used in the following experiments.

The spores were exposed to the treatment in four different ways: (1) Three drops from a cloudy water suspension were placed on the bottom of a sterile petri dish with a sterile platinum loop and dried before treating. (2) Three drops of the suspension were placed in a dish as above and the dish was placed in the treating chamber before the drops had dried. (3) The drops from the suspension were placed on sterile cover glasses and these were then placed in sterile petri dishes and treated. After treatment the cover glasses were removed to another petri dish in order to avoid a chance of getting any great amount of formaldehyde into the culture medium when the plates were poured. (4) Masses of dry spores were used. The masses of spores were placed on cover glasses by smearing with a platinum loop containing an abundance of spores taken from the surface of a pure culture. These cover glasses were then handled as described under the third method.

The checks were made in the same way as the plates used in the first method except that they were not treated in any way. After the treatments all plates were poured, using potato agar. They were kept under observation for from a week to 10 days.

The use of different amounts of formaldehyde solution and changes in duration of the treatment show that under the conditions described 10 ounces of standard formaldehyde solution per 1,000 cubic feet for 1 hour will kill the organisms used when they are exposed in a thin film. When a mass of spores is used, more time is necessary to kill them.

The masses of organisms or spores in the case of *Bacillus caratovorus* and Monilia were killed when formaldehyde was used at the rate of 10 ounces per 1,000 cubic feet for 2 hours; Ascochyta spores in mass were killed when formaldehyde was used at the rate of 20 ounces per 1,000 cubic feet for 1 hour; but 20 ounces per 1,000 cubic feet for 2 hours was necessary to kill the masses of spores of Colletotrichum. Fusarium proved to be the most resistant, and a test was made using the spores of four different species of Fusarium in masses. Formaldehyde was used at the rate of 30 ounces per 1,000 cubic feet for 2 hours, and in all cases growth occurred.

Following the above experiments some work was undertaken to determine the effect of formaldehyde vapor on the fungous spores and bacteria borne on the surface of seeds. Five seeds of each of the various kinds

used above were placed in a series of sterile petri dishes, two sets of each kind of seeds being used. One set of dishes was held untreated as a check and the other set was given the vapor, using formaldehyde solution at the rate of 20 ounces per 1,000 cubic feet for 2 hours. The plates were all poured in the usual way and observations were made for several days. This experiment was repeated three times, and very promising results were secured. Alfalfa, carrot, clover, field pea, flax, lettuce, millet, muskmelon, radish, and soy beans were free from fungi when treated, but fungi were present in abundance in all the checks, except flax.

In practically all cases, whether treated or untreated, a few bacteria developed on the plates, but the treated plates showed very few colonies, while the checks showed a great many.

In the case of barley, oats, corn, rye, rice, and wheat no growth appeared for two or three days in the treated plates, while the untreated plates had an abundance of growth within a day or two. This difference may be due to an inhibiting effect on the part of the vapor, but it seems more probable that it is due to the fact that the surface spores and mycelia were killed in the case of the treated seeds and that the appearance of fungi a day or two later is due to the growth of mycelium from within. The fungi appearing in such cases were species of Fusarium and Alternaria.

It seemed desirable to determine in so far as possible what fungi are present on the seeds passing through the inspection house and at the same time get some additional information as to what effect the vapor treatment would have on these organisms under actual working conditions. A chance was also afforded to study the effect of the vapor treatment, in a very limited way, on germination.

In this work five seeds of the material to be tested were removed before treatment and five after treatment. These seeds were placed in sterile petri dishes and treated in the usual way. The plants were under observation for several days. Bean seeds were used more than any other, inasmuch as a large number of shipments of beans happened to be coming in from South America.

Out of the 86 different samples of beans treated and studied 9 seemed to be retarded from one to two days in germination, while 8 were accelerated slightly, but in no case was there any apparent injury. The remainder of the samples did not seem to be affected one way or the other, so far as their germination was concerned. There was very marked reduction in the number of fungi and bacteria present in the treated samples as compared with the untreated. In taking samples of this kind, average seeds were selected, and as a result some of the beans were diseased and probably had internal mycelium, as in the case of Colletotrichum, thus making it impossible to render them absolutely free from fungi without killing them.

Sixteen different fungi were found in these samples, including Fusarium, Alternaria, and Colletotrichum, species of all three of which are known to cause serious bean diseases.

In all of the experiments set forth above, only a few seeds were included in each sample and inasmuch as formaldehyde vapor is known to be lacking in penetration it seemed desirable to try the treatment of a larger quantity of seeds.

A shipment of poppy and Cryptotaenia seed afforded an opportunity to try the treatment of a larger quantity of seeds than previously had been attempted. The samples used were of sufficient size to cover the bottom of the dish in which the seeds were treated to a depth of three-fourths of an inch. After treating the seeds a sample was taken from the surface and then the seeds were carefully removed from the surface to a depth of about one-half inch where another sample was taken. These samples were plated out and the samples taken one-half inch below the surface showed fully as many colonies of fungi and bacteria as did the untreated samples while those taken from the surface showed no fungi and a marked reduction in bacteria. This experiment shows the lack of penetration of formaldehyde vapor.

A comparison of the formaldehyde vapor and a 2 per cent formalin solution was made. Ten different kinds of seeds were used and three samples, each consisting of five seeds, of each of the different kinds of seeds were made and placed in sterile petri dishes. The first set of each was retained as a check; the second was treated with 2 per cent formalin for 10 minutes and then washed with sterilized water twice, while the third was given formaldehyde gas at the rate of 20 ounces of formalin per 1,000 cubic feet for 2 hours. After treatment all the plates, including the checks, were poured and kept under observation for several days. In all cases the 2 per cent formalin sample stood intermediate between the checks and those treated with formaldehyde vapor. The vapor-treated samples were remarkably free from fungi and bacteria. In fact only the wheat, rice, and rye samples had any fungi present, and there was much less growth in these than in the checks or in those treated with 2 per cent formalin. One of the noticeable things in this experiment was that in all the plates treated with the vapor there was a very marked reduction in the number of bacterial colonies, as compared with the checks, while the samples treated with 2 per cent formalin showed little or no reduction in the number of bacterial colonies, as compared with the checks It should be stated in connection with these experiments that different lots of seeds or a change in any one of the many factors concerned in all probability would bring about a change in the results obtained.

Several hundred lots of seeds have been treated with 20 ounces of formaldehyde per 1,000 cubic feet. Subsequent plating in agar has shown that molds and other fungi rarely appear in these plates if the

seeds are sound, whereas the untreated checks seldom fail to develop several colonies.

The work thus far necessarily has been limited to a few fungi and a few seeds, but there is an almost unlimited field here that needs investigation if efficient and satisfactory results are to be obtained in the disinfection of seeds.

CONCLUSION

1. The use of liquids for disinfection is unsatisfactory for many kinds of seeds.

2. A number of species of fungi and bacteria are killed when treated for 2 hours with 20 ounces of formaldehyde vaporized under the conditions described.

3. This same treatment is not injurious to any of the seeds tested.

4. The experiments completed indicate that the formaldehyde gas treatment described is a very efficient means of seed disinfection.

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INFLUENCE OF SOIL ENVIRONMENT ON THE ROOT-ROT OF TOBACCO

By JAMES JOHNSON, *Assistant Professor of Horticulture, College of Agriculture, University of Wisconsin*, and R. E. HARTMAN, *Agent, Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture*[1]

COOPERATIVE INVESTIGATIONS OF THE OFFICE OF TOBACCO INVESTIGATIONS BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE AND THE WISCONSIN AGRICULTURAL EXPERIMENT STATION

INTRODUCTION

The foremost considerations in connection with the study of disease in plants are the pathogenicity of the parasite, the susceptibility of the host, and the environmental conditions favoring the infection and progress of the parasite. It is well known, however, that the relative pathogenicity of the parasite and susceptibility of the host are not always easily distinguishable one from the other in disease, and that they are largely influenced by environmental conditions. To the practical grower environmental conditions have been considered as all important, to the exclusion of the parasite, while, on the other hand, the tendency in the past on the part of pathologists and botanists has been to devote a great deal of energy to the study of the parasite, with only passing interest being given to the influence of the environment on disease, as recently emphasized by Jones (*16*).[2] This is especially true of plant diseases having their origin or region of attack on underground portions of plants. The literature upon actual experimental data with reference to the influence of soil conditions upon a soil-infesting parasite is fragmentary, and for the most part concerned with only one or two variable factors, so that the conclusions can not always be relied upon because of failure to give due consideration to other factors perhaps even more influential in the end result obtained. The Thielavia-rootrot of tobacco (*Nicotiana tabacum*) forms a relationship of host and parasite apparently admirably adapted for such experimental work in that it permits quantitative determination of the influence of the disease upon the host;

[1] The writers are indebted to Dr. L. R. Jones, of the Department of Plant Pathology, Wisconsin Agricultural Experiment Station, for helpful suggestions, and to Dr. W. W. Garner, of the Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture, for critical reading of the manuscript.

[2] Reference is made by number (*italic*) to "Literature cited," p. 85-86.

the fungus is readily recognizable, and both the parasite and the host are easily manipulated under widely varying environmental conditions. With reference to this disease alone no problem was seemingly more in need of investigation from a practical standpoint than the great variability in the occurrence of the disease observed both in general and local areas, and the influence of external conditions on the application of prophylactic measures. The literature, furthermore, abounds in statements intended to explain the epidemics of this disease, which are greatly in need of modification and correction.

Accordingly, a study was undertaken with the view of covering practically all phases of the environmental conditions which might influence the tobacco rootrot. Although it is felt that the problem is still in need of further study, it is believed that the evidence here presented will serve to show the relations of the more important factors concerned.

SYMPTOMS OF ROOTROT

The rootrot of tobacco and other plants, caused by *Thielavia basicola* (B. and Br.) Zopf, is the most serious disease with which the tobacco growers in most producing sections have to contend. Its importance is especially evident in Kentucky, Wisconsin, Ohio, Connecticut, and Pennsylvania. The aboveground symptoms are much the same as those produced by the usual unfavorable soil or weather condition which may stunt the growth of tobacco; hence, as a rule, its effects are not recognized by the growers as having a parasitic origin. Where infection is abundant, however, the signs of the disease on the roots are sufficiently specific to leave no doubt as to the causal organism. It is difficult, however, even for the pathologist to judge adequately the relative amount of damage done by *T. basicola* and by other causes which may reduce yield, even when the roots are carefully removed from the soil and washed before examination. The relative importance can be determined with considerable accuracy, nevertheless, by comparing the plants especially the roots, which have been grown in infested and uninfested or sterilized soil; or by comparing both resistant and susceptible strains grown on infested soil. In this way one may find what appears to be a comparatively unimportant amount of infection is in reality a controlling factor; or, on the other hand, that a seemingly heavy infection is of comparatively small importance. In this way casual judgment may be replaced by definite experimental evidence.

The effects of the rootrot may range from a complete checking of the plants, or even death when infection occurs in the early stages of growth, to only slight signs of reduced yield. It is indeed highly probable that under certain conditions considerable infection may be present without appreciably affecting the yield. Furthermore, it appears to be equally certain that in some instances infection by *T. basicola* has markedly increased yields as a result of temporarily delaying growth during a period un-

favorable for normal plant growth, hence preventing maturity of the crop until seasonal conditions develop more favorable for the normal growth of the host, but at the same time unfavorable for the development of the parasite. On the other hand, the parasite has produced through this indirect action heavy losses due to hail or frost injuries, or has reduced the quality of the product as a result of extending the ripening and curing process into unfavorable seasons. No above-ground symptoms of rootrot are more common than the failure of tobacco to grow appreciably during the first month or six weeks after transplanting to the field, followed by a period of relatively rapid growth and development caused by a change of conditions which have up to the present remained more or less obscure.

No detailed description of the disease on the roots need be given here, as this phase of the subject has been frequently presented and will become more or less evident in the progress of the present discussion.

REVIEW OF THE LITERATURE

Peglion (*20*) was the first to describe *T. basicola* as a parasite of tobacco when he reported it from Italy in 1897. There is considerable evidence, however, that this disease had occurred upon tobacco in America many decades prior to that time, although it was not recognized as a disease. When Jones, according to Tatham (*24*), as early as 1724, wrote with reference to agriculture in Virginia—

> when land is tired of tobacco, it will bear Indian corn or English wheat or any other European grain or feed with wonderful increase—

he made a statement which is being annually "rediscovered" by hundreds of tobacco growers, but which is an established principle with thousands of other growers. It is now quite certain that parasitism explains the majority of the modern tobacco growers' difficulties of the nature referred to above, and no hypothesis yet formulated will explain with equal satisfaction the observations of the early Virginia planters.

Antedating the first report of the parasitic origin of the rootrot by 13 years, Killebrew (*17*) in 1884 wrote:

> In some years the plants both in the seed bed and after being set out are affected by a disease known as the "black-root." The plants so affected do not die, but after standing comparatively still for a long time revive later in the season, but do not make a good quality of tobacco. It is not known what the agencies are producing this disease, nor has there been a remedy discovered for it. By some it is believed to be the result of sowing seed continuously in old beds. Seed beds in newly cleared ground are said to be entirely free from it.

There can be no doubt that this is the description of the rootrot, or blackrot, of tobacco caused by *T. basicola*. This brief description of the disease is given in full, since it is probably not only the first authentic report of the disease but also because it describes the common behavior of infected plants, as follows:

> after standing comparatively still for a long time revive later in the season.

This observation may be repeatedly noted in infested soils, and it was with the idea of explaining this condition particularly that the investigation on the influence of environmental conditions upon the disease was begun in 1914.

Sorauer (23) in 1895 made some observations upon the rootrot of cyclamens, and concluded that heavy manuring, too abundant watering, and too high temperatures favored the attack by the parasite.

Peglion (20), who first reported the disease on tobacco in 1897, also believed that too much manure and water were controlling factors in producing the disease.

Campbell (5) believes excessive quantity of humus in the soil predisposes the tobacco plant to disease, and also that an acid condition of the soil weakens the plant and predisposes it to disease.

Buttaro (4), probably following the lead of other European authors, also writes that the disease on tobacco is favored by abundant organic matter, excessive humidity, and high temperatures.

Benincasa (1) concludes that in some years the disease appears only slightly or not at all, and states that its development is favored by too much organic matter, excessive watering, and generally damp weather. Benincasa, at about this time, began to study the relation of moisture and different kinds of soils to the development of the disease. He concluded at this time that porous soils give the best results and in 1911 he discussed the subject in more detail.

Cappelluti-Altomare (6) concluded that the disease could be checked by limiting the amount of watering and by not reducing too greatly the light and air supply of the seed beds. He also advises against sowing the seed too thickly.

Galloway (11, pp. 174–175) reporting on the wilt of violets, caused by *T. basicola*, advises against the use of decaying vegetable matter in the propagating beds. His statement that—

> plants affected may make a good growth in summer and show no evidence of trouble until September or October, when they will wilt more or less during the day and revive at night.

is especially pertinent to the investigations in this paper.

Clinton and Jenkins (9) suggest that excessive fertilization, soil reaction, and soil moisture, the latter in particular, may be important secondary factors determining the extent of the injury by *T. basicola*. They also state that the cold, wet weather of early spring helps along the trouble in the seed beds, particularly when they are not properly ventilated.

Clinton (8) is quite convinced that the character of the season, especially the moisture and possibly unusually cold wet spring weather, and the character of the soil and subsoil—fineness, liability to become water-soaked, drainage, amount of humus, especially in the shape of manure—have much to do with determining whether or not the fungus

does much damage. No definite experimental data are given, however, in support of these views. The following year (1908) drouth is said to have reduced the injury due to the disease.

Briggs (*3*) reported, upon evidence obtained from Connecticut soils that the fungus attacks are most severe on soils made alkaline by large applications of lime, ashes, or fertilizers containing carbonate of potash, and that the alkaline condition in infested soils should be corrected by the use of acid fertilizers in order to obviate the damage by *T. basicola*. This advice was received favorably by both practical growers and scientists, and many recommendations were based upon it.

Gilbert (*12*) concludes that an abundance of humus, a considerable percentage of clay, high fertilization either with chemicals or manure (especially nitrogenous fertilizers), excessive water, and high temperatures favor the disease. In an experiment to determine the influence of the amount of watering he found that excessive water increased the disease, although 62 per cent of the plants in the scantily watered beds were diseased. He also compared the yield as a result of transplanting diseased and healthy plants in the field, using a "Havana Broadleaf" variety. He obtained as good yield from the diseased plants as from the healthy ones.

Whetzel and Osner (*27*) recommended acid-phosphate fertilization for the control of *T. basicola* which causes fiber-rot on ginseng.

Benincasa (*2*) reporting on results obtained in comparing different "soils" for growing tobacco plants, recommends sand or "*pozzolano*" a volcanic ash for this purpose, since favorable conditions for disease are said to be absent in these . He also states that *T. basicola* is a weak parasite under certain conditions.

Martinazzoli (*18*), however, reported that he obtained *T. basicola* from beds where *pozzolano* was used, infection probably having come from soil.

Massee (*19*) concludes that *T. basicola* can not infect host plants in pure sand, since the fungus is able to infect only in the presence of organic matter which will permit the mycelium to exist for some time as a saprophyte.

Chittenden (*7*) had difficulty in obtaining infection with *T. basicola* until overwatering of the soil was practiced.

Rosenbaum (*22*) believes that such external conditions as excessive water, lack of aeration, and heavy manuring favor infection.

Reddick (*21*) reported unsatisfactory results for the control of Thielavia of violets by acidifying the soil with acid phosphate as recommended by Briggs. Stable manure apparently did not act deleteriously on infested soil. The experiments were not carried far enough, however, to be entirely conclusive.

The present writer (*14*), as a result of field observation, also believed soil moisture to be the main controlling factor in determining the severity of the disease.

GENERAL CONSIDERATION OF FACTORS CONCERNED

It is evident at the outset that any attempt at a separate analysis of each factor concerned in disease occurrence is practically impossible. Varying one single factor of the environment to the total exclusion of variability in all others is an ideal to be kept in mind in experimental work of the nature to be described. Failure to reach this ideal in practice, however, need not necessarily reduce the value of the result, provided the effect of other variables on such a result is properly considered. As an illustration of a complication of factors of this sort, there may be cited the maintenance of two pots of soil at two different temperatures, say 30° and 10° C., respectively, in order to compare the effects of these temperatures on the occurrence of *T. basicola* on the roots of tobacco. By means of proper controls in uninfested soil the influence of many factors involved may be eliminated, but it does not seem possible to separate clearly the factor of soil temperature from that of soil moisture. The soil and plants at 30° will require several times as much water as will the soil and plants at 10° because of the increased evaporation and transpiration at the higher temperature. The correct replacement of this water for maintaining like moisture relations for the host and parasite is uncertain no matter how frequently and carefully it may be done either by weight or by the use of an auto-irrigator. If, however, the moisture relations have previously been studied and the range of the effects to be expected from this factor are known, it may be possible to carry on soil-temperature studies with only moderate attention to the moisture relations.

A study of the factors concerned in the development of the Thielavia rootrot has served to bring out clearly the fact that all the factors concerned are inseparably connected with one another, and that the amount of disease occurring is the product of a number of plus and minus factors, but that, nevertheless, in an analytical study of this nature, it is possible to arrive at the relative importance of these various influences.

It is important, furthermore, that not only the true environmental factors be taken into account, but also that such inheritable factors as the relative degree of susceptibility of the host plants used and the virulence of the parasite concerned receive proper consideration. In addition, the amount of infection and the time of its occurrence may greatly modify the results both as regards the readiness with which the host may become infected and the effect of a "mass action" upon the measurable end result of disease.

The purely environmental conditions to which the roots of the host or the parasite harbored by the soil are subjected may be conveniently considered under the following subjects: (1) Amount of infestation present; (2) percentage of moisture; (3) temperature; (4) soil reaction; (5) physical

structure of the soil, including the relative amount, the state of the vegetable matter, and the size of the mineral particles; (6) available chemical fertility; (7) state of cultivation as regards compactness and aeration.

To the soil physicist, chemist, and biologist it will appear that the entire scope of soil science may be concerned in the production of disease in the roots of plants, and such seems to be the case. On consulting the branches of soil science it is at the same time both encouraging and discouraging to find many of these factors influenced by a number of other interrelated factors under normal conditions. For instance, soil temperature as such can not be thought of without also considering the influences of the air temperature, specific heat, moisture content, exposure, and color of the soil upon such temperature. With soil moisture it becomes essential to regard moisture-holding capacities, rainfall, drainage, cultivation, humidity, and temperature; or, in the case of soil fertility, to consider along with the natural fertility, its cropping history, applied fertility, and various other modifying factors.

With these things in mind, however, it has become increasingly possible to account for, if not to explain fully, seeming contradictions and lack of accord with established principles of infection which have come under the writer's attention during the past five years in the case of the rootrot of tobacco. The occurrence and economic importance of the disease in one State and not in another, on one farm and not on the neighboring farm, or on the hilltop in one field and in the low spots of another, as well as the total failure of a crop in a field one year followed by a complete success the following year, or the change of crop prospects from failure to 100 per cent yield within the period of two weeks, are all more or less subject to scientific interpretation from this viewpoint.

With respect to those factors, aside from environmental conditions which may influence experimental results, it should be said that as far as evidence from literature, or as far as the observation of the writer is concerned, there is nothing to indicate that specialized races of *T. basicola* occur, or that the fungus varies in any way in virulence owing to differences in strain or age of cultures. It may be said with considerable certainty, therefore, that we are dealing with a relatively constant organism as to pathogenicity. With respect to host differences it has been shown (*13*, *14*) that very decided differences in susceptibility in host plants, and in varieties and strains of tobacco occur. By using pure strains of seed experimental error from this source may be eliminated. It should be remembered, however, as will be shown in the data here presented, that because of these differences in susceptibility the critical points in disease occurrence and severity may be shifted in one direction or another to some extent, a fact which makes it important that the susceptibility of the variety used for experimental work be taken into account in any interpretation of results.

AMOUNT OF INFESTATION

With diseases in which the injury from infection is confined to relatively small local areas on the host, it is to be expected that the amount of damage done is in some measure proportional to the number of infected areas which occur. Although it is evident that under the most favorable conditions, *T. basicola* may spread a considerable distance over a single root from one source of infection, more commonly the diseased portions are confined to areas of from 1 to 5 mm. In either case it is evident that relatively few infections may cause no appreciable stunting in growth, where a larger number of infections in proportion to the size of the root system may produce a rapid check in growth, owing largely to the mechanical reduction of the feeding area, and possibly also in some degree to the formation by the fungus of toxic substances injurious to normal metabolism. The latter possibility appears not to hold, however, since the host is rarely killed by the parasite. In fact, the plant appears to receive a stimulus toward the formation of new roots to replace those lost by disease, and in many instances the youngest leaves of infected plants possess a deeper green color than healthy plants. The new roots are at a greater disadvantage than the original ones, if they are formed at or near the region of disease, since frequently they are obliged to pass through small local areas of high infestation before reaching the deeper layers of soil.

It is a well-recognized fact that infestation increases under field conditions at an enormous rate once it is present or introduced to a soil which is being cropped to a highly susceptible host. This is illustrated in the practice in certain tobacco-growing areas of growing only one or two crops of tobacco and then laying the land aside for other crops for a long time. That this practice has been empirically developed, in the Burley section at least, as a result of the rootrot, seems unquestionable, in view of some unpublished results obtained in that section during the last three years.

The influence of the amount of infestation on the amount of disease might be illustrated in a number of ways. The simplest method appeared to be the mixing of varying quantities of infested and uninfested soil and transplanting into this mixture a susceptible variety of tobacco. The soil selected for this purpose was from the old tobacco field on the Station farm at Madison, on which tobacco had been grown continuously for 10 to 12 years, together with soil of the same type from a neighboring plot located not more than 2 rods away, but never having been used for growing tobacco. The fertility of the two soils naturally would not be the same, but the soil from the tobacco field because of heavy applications of fertilizers was the more fertile of the two from a chemical standpoint. The soils, after having been thoroughly screened, were weighed out and mixed in the proportion shown in Table I. Two sepa-

rate experiments of this nature were carried out. In a third experiment, steam-sterilized tobacco-field soil was used for mixing with the untreated tobacco soil in the same way as before. A gradual falling off in growth will be noted with the increase of amount of infested soil (Pl. 1, I). In the case of No. 4, in experiment 3, the lowered yield, as compared with all infested soil is no doubt due to the frequently observed fact that reinfested sterilized soil favors the growth of fungi and consequently the increased development of disease in the soil. In a mixture of three-fourths infested and one-fourth steamed soil the balance of infestation and conditions apparently was such as to cause greater infection than in all-infested soil.

TABLE I.—*Influence of amount of infestation of soil by Thielavia basicola on the yield of tobacco*

Pot No.	Soil mixture.		Average air-dry weight of duplicates.		
	Infested.	Uninfested.	Experiment 1.	Experiment 2.	Experiment 3.[a]
			Gm.	*Gm.*	*Gm.*
1	None	All	3.25	5.37	6.93
2	One-fourth	Three-fourths	1.65	3.66	4.20
3	One-half	One-half	1.40	2.73	2.65
4	Three-fourths	One-fourth	.70	2.64	[a] .51
5	All	None	.01	1.32	1.33

[a] Steam-sterilized soil was used as uninfested soil in Experiment 3. The low yield in pot 4 was probably due to steamed infested soil favoring disease more than unsteamed infested soil.

The results obtained in Table I are considered to be due only to the fact that more spores exist per unit of soil in the higher proportion of infested soil, and therefore the roots are more likely to come in contact with infecting material. This fact has an important bearing upon results obtained in a study of environmental conditions. However, since any deleterious or beneficial action to which the parasite is subjected may merely reduce or increase the amount of infestation, the results will be proportional in some measure to the amount of infestation present.

INFLUENCE OF MOISTURE CONTENT OF SOIL

A review of the literature concerning factors influencing the severity of the rootrot of tobacco shows that soil water has been considered the primary limiting factor by practically all observers and investigators of this disease. Most of the conclusions drawn in regard to this, however, have been based on observations in the greenhouse or in plant beds, where artificial watering is resorted to and where it is relatively common in many instances to overwater the soil. A study of the disease under field conditions with reference to moisture, though equally indefinite and inconclusive owing to the number of other variable factors, is at any rate convincing that an oversupply of moisture is not necessary for heavy infection and severe attacks by the parasite.

In order to get more accurate evidence on the influence of soil moisture, a series of pot experiments, with the moisture supply controlled as closely as possible by weight, were carried out.

Several difficulties, not readily overcome, exist in such an experiment, the principal one being that it is practically impossible to maintain a uniform moisture content throughout the soil. No doubt the use of Livingston auto-irrigators would have made possible more uniform results, but these were not available at the time. Two-gallon crocks, perforated at the base for drainage and holding about 10 kgm. of soil, were used. The naturally infested soil from the old tobacco field on the Station Farm was used. After a large quantity of this soil had been dried, thoroughly mixed, and screened, its moisture content and water-holding capacity were determined in the ordinary manner. Ten kgm. of the soil were then placed in each of twenty 2-gallon crocks. The soil in 8 of these crocks was sterilized by steam at about 100° C. for the purpose of destroying all the *T. basicola* present in order to provide disease-free controls in the experiments. The water relations, as well as the food relations, were, of course, changed in some degree by the sterilization, and an absolute comparison between the sterilized and infested series was therefore not permissible, although it is believed that the results are not altered appreciably by this fact.

The crocks of soil were then divided into four series, each containing three infested and two uninfested crocks of soil. Two glass tubes, ½ inch in diameter, one being inserted to a depth of 2 inches and the other to a depth of 6 inches, were placed in each crock for the purpose of permitting a more uniform distribution of water in the soil. Of the four series, one was now made up to one-fourth its full water-holding capacity, and the others to one-half, three-fourths, and full water-holding capacity. After the water had been allowed to distribute itself fairly evenly, one plant of the White Burley variety grown in sterilized soil was transplanted to each of 20 crocks. The loss of moisture from the crocks was very slow when the plants were small, especially during the winter in the greenhouse. Usually it was not necessary to make the pots up to the required weights oftener than once every three days, but later in the tests daily attention was usually necessary. In an experiment begun on February 13, 1917, with the White Burley variety, it was noted at the end of one week that in the infested series the plants at one-fourth saturation wilted during days of high transpiration and showed the poorest growth. The plants at three-fourths saturation got the best start, while those at full saturation were already yellowing and apparently diseased, since no such condition was observed in the sterile controls. On March 5 the conditions were about the same in relative growth except that the diseased condition of the plants at full saturation in infested soil was greatly increased, and the controls in sterilized soils were now beginning to forge rapidly ahead of those in infested soil. On March 14 it seemed quite

evident that in uninfested soil the optimum moisture content of this soil type for the growth of tobacco lay close to three-fourths saturation and that full saturation was more favorable than the one-half and one-fourth saturation. Although still no great differences existed between the infested and uninfested soils at one-fourth, one-half, and three-fourths saturation, the plants in the uninfested soil at full saturation were about 10 times as large as those in the infested soil at the same saturation.

The data taken upon the growth of the plants in these experiments are recorded for the most part as the total leaf area of each plant taken at intervals of about one week. These determinations were made by placing the leaves over a standard leaf-area chart on which areas for varying sizes and shapes of leaves had been previously determined with a planimeter. These areas, expressed in square inches, were determined at the end of this experiment, on March 28, and are given under experiment 2, Table II. The results appear to justify the conclusion that a fairly constant ratio exists between the growth on infested and uninfested soil at the three lower saturations. This ratio is approximately 1 to $3\frac{1}{2}$. On the soils at full saturation, however, the ratio of growth on infested soil to that on uninfested soil is about 1 to 40. The evidence from this experiment therefore shows that a very considerable amount of disease can occur in a relatively very dry soil and that it does not appear to be proportionately increased in a relatively moist soil, but that a wet or saturated soil, which still permits a good growth of tobacco when uninfested, causes a rapid decrease in yield when infested with *T. basicola*.

TABLE 2.—*Influence of the moisture content of the soil on the amount of tobacco rootrot*

Saturation.	Approximate percentage of moisture.	Experiment 2: Average leaf area (square inches).		Experiment 4: Average air-dry weight (gm.).	
		Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.
One-fourth	7.7	34	9	2.0	1.5
One-half	15.5	81	22	9.8	4.5
Three-fourths	23.3	301	89	19.7	5.4
Full	31.0	239	7	9.3	1.1

In a following experiment, which was conducted on the same soil in the greenhouse, the above results were practically duplicated so that the data will not be presented here. A third experiment was conducted during the growing season in a shelter out of doors, permitting atmospheric relations more nearly normal than those occurring in the greenhouse during the winter season. The experiment was run in the same manner as the foregoing one, except that a change of soil was made, another batch from the same source was used in order to avoid nematode injury. The crocks were set to White Burley on July 5. On July 12 all the plants appeared to have a good start except those at one-fourth

saturation, which usually wilted during the daytime owing to lack of moisture. By July 21 marked signs of heavy infection of all the plants in the infested soils was shown by reduced growth and yellowing of the lower leaves; this condition was most marked at full saturation. The uninfested soil showed the optimum growth at three-fourths saturation. On August 14 the experiment was discontinued, the plants photographed (Pl. 1, II–III), and then cut and dried. The air-dry weights are given in Table II under experiment 4.

While the results of this experiment as shown by air-dry weight in comparison with the leaf area given in experiment 2 do not conform in all details with those of experiment 2, they are believed to agree in general in that the greatest amount of injury from disease resulted in the saturated soil; the ratio of the yield on infested soil to that on uninfested soil was again considerably greater than in the other cases.

It is realized that further experimental evidence could be profitably obtained as to the relation of moisture to the disease. The difficulties already referred to, however, together with complication of other factors such as temperature, and some of the more obscure factors such as aeration and compactness of the soil, have rather discouraged further tests until more accurate technic can be devised. It is certain, however, that T. bascicola has a wide range of action as regards actual percentage of moisture present in the soil. It has been found, for instance, that in water culture containing a spore suspension, good infection occurs on roots and that it will occur in soils too dry to permit anything like normal growth of tobacco. Whether there is a direct increase in amount of infection and severity of the disease with percentage increase of moisture in the soil may not be exactly clear from the data here presented. From the majority of the data obtained, however, some of which is not given here, it seems fairly certain that such direct proportionality does not exist, but that a fairly constant relation is maintained in soils with moisture content ranging from those sufficient only for poor plant growth to those approaching saturation, followed by a very rapid increase of disease from this latter point up to full saturation.

At any rate it may be said that poorly drained infested soils which are likely to remain saturated for a period of three or four days, or any infested soil kept near saturation for a period of days due to excessive rainfall will undoubtedly show higher infection than well drained soils or soils not affected by an excessively wet season. On the other hand it appears that, as a rule, soil moisture is not an important controlling factor in the prevalence of the rootrot of tobacco. Relatively dry or relatively wet seasons, in so far as they affect soil moisture alone, are not especially to be feared nor to be relied upon for holding the disease in check. For the same reason a careful check has not been kept upon the moisture content of the soils in the various experiments described

in this paper with other environmental conditions. The soils have been kept as nearly as possible uniformly watered, never approaching saturation for any considerable period of time and never relatively dry, so that it is not believed that the results have been vitiated by this factor, though it is admitted that they may have been responsible for many of the individual variations in results always occurring in experiments of this sort, and which is planned to be overcome by mass of data rather than by the most careful attention to a single experiment.

If the above-ground symptoms of the rootrot are considered, it is at once realized that the reduction of the water supply is probably the most important one, partly as a result of reduced food supply to the plant brought about by the gradual but effective depletion of the root system. It is therefore usually quite impossible to judge from the above-ground portion of plants alone as to whether lack of available soil moisture or lack of roots or both are responsible for a reduced yield. It is only when growers obtain a greatly reduced yield on land known to be in a high state of fertility that they begin to suspect other troubles. It is said with confidence, therefore, that fully nine-tenths of the damage by the rootrot is attributed by the growers either to a deficiency or to an excess of soil moisture. Sixteen moisture determinations of the field plots (the same soil as used in the pot experiments) at about 3-day intervals between June 19 and August 6 in the summer of 1917 showed a range of moisture content from 25.3 per cent on June 25 to 16.2 per cent on August 2. These determinations showed that during the entire season the moisture content was practically between the limits of one-half to three-fourths saturation. In these plots White Burley tobacco made no growth whatever during this time because of *T. basicola*, and Connecticut Havana made only half a crop. It is clear that the moisture content was not excessive for the best growth of tobacco, and yet the parasite was almost at its maximum of activity.

INFLUENCE OF SOIL REACTION ON ROOTROT

The reaction of the soil has been considered to play a part in the severity of parasitism in practically all plant diseases having their origin in the soil. The reasons for these rather widespread calculations are perhaps manifold. Among the earliest chemical agents applied to soil with the hope of checking plant pests was lime, and experiments too numerous to mention here have since been conducted with it in the hope of checking the diseases and insects attacking plants. Where lime has proved efficacious, however, pathologists have considered it both as influential as a sterilizing agent against the parasite and as a neutralizer of soil acidity favorable to parasitic action. The beneficial action of lime to the growth of green plants and to bacterial activity in soils has also no doubt served to stimulate its use in phytopathological problems. No advantage is to be gained by reviewing the rather extensive study of

the value of lime in the control of numerous plant parasites harbored in the soil because the results obtained depend altogether upon the disease concerned. Experiments in its use have been most complete in relation to the control of potato-scab, clubroot of crucifers, and nematodes. Limed soils favor scab, whereas clubroot and nematode injury are much reduced by its use.

With the appearance of a paper by Briggs (*3*), based on field experiments in Connecticut, a great deal of interest was revived on the influence of soil reaction on plant diseases having their origin in the soil. Briggs concluded briefly that materials applied to the soil which tended to make it alkaline in reaction favored Thielavia-rootrot of tobacco, whereas materials applied which made the soil acid reduced the disease. The actual change in soil reaction apparently was not determined. On the basis of these results the use of acid fertilizers came to be recommended in both scientific and popular literature and the use of lime cautioned against. Considerable experimental work also was undertaken at various places with this and other diseases, some of which apparently verified the results of Briggs, while others showed no favorable results. Thus, the problem has remained in a more or less uncertain state. Clearly it is not one which is easily solved. Changing the reaction of the soil from acidity to alkalinity, and especially from alkalinity to acidity by the application of different chemicals, is open to many difficulties not clearly analyzable. The problem of soil reaction as influencing disease presents two aspects: First, to determine the actual influence of the reaction of the soil medium upon the disease; and second, to determine in how far this influence may be utilized in a practical manner by actually changing the reaction of large areas of soil to a sufficient degree to modify the severity of the disease. The latter problem is complicated by a number of factors, the most evident of which is the naturally or normally attained reaction of the soil, since this must have considerable bearing upon the amount of acid-producing materials which must be applied to get the desired result. Aside from the final influence of such treatment on the soil itself, in a system of economical and permanent agriculture a more discouraging feature of the problem is that from the standpoint of the disease, for, as will be shown, many other factors must be taken into consideration, such as the susceptibility of the variety of tobacco grown, the amount of infestation, and the temperature of the soil. By varying these factors markedly different results may be secured on the influence of soil reaction in relation to disease.

The experiments carried out in the investigation presented here were of two kinds, pot tests and field plot tests. The former were carried on for the most part in the greenhouse during the winter season and the latter on an old heavily infested tobacco field on the Station farm at Madison.

POT EXPERIMENTS

As has already been suggested, it is especially difficult to render an alkaline soil acid in various degrees by the application of a theoretical quantity of an acid or acid salt. The alteration produced in the soil by either treatment is likely to alter it so fundamentally that comparison with another soil treated in a dissimilar manner tends to complicate the results to an unnecessary degree. What seems to be a considerably better plan is to select a naturally highly acid soil and to change its acidity to various degrees of alkalinity by the application of the theoretically correct quantities of lime. Accordingly, this plan was followed.

The soil selected was a very acid Sparta sand from a field at Lavalle, Wis. A total acidity determination of this soil by the Truog method (*26*) showed that its lime requirement was 9.38 tons per acre. The strength of acidity was found to be 108 on the basis of acetic acid at 1,000. The soil after being finely screened was placed into 2-gallon stoneware crocks, perforated at the base for drainage. Ten kgm. of soil were weighed into each of 36 crocks. These crocks were then divided into 9 sets of 4 crocks each. The calculated quantity of precipitated calcium hydroxid of the highest purity was thoroughly incorporated in the soil of each of the 4 crocks in each series with a view to reducing the acidity to fairly definite degrees. In Table III are given the quantities of calcium hydroxid applied, together with the determinations of total acidity by the Truog method made several months later, when it was considered that the full effect of the treatment on the soil had occurred.

TABLE III.—*Influence of soil reaction on development of rootrot*

Series.	Quantity of lime added to 10 kilograms of soil.	Lime requirement per acre.	Average air-dry weight.						
			Experiment I (White Burley).			Experiment II (Connecticut Havana).		Experiment III (Maryland Broadleaf).[a]	
			Infested soil.	Uninfested soil,	Amount of infection on roots (infested soil series).	Infested soil.	Uninfested soil.	Infested soil.	Uninfested soil.
	Gm.	*Tons.*	*Gm.*	*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*
1.....	0.0	9.38	2.46	2.72	Very slight.....	6.35	6.45	0.11	6.80
2.....	17.80	7.19	1.09	4.09	Considerable.....	2.65	5.95	.11	5.00
3.....	35.60	4.60	.33	4.65	Heavy.....	2.22	5.82	.18	8.15
4.....	53.40	2.62	.17	4.40	.....do.....	.75	8.20	.14	8.67
5.....	71.25	.72	.68	1.49	.....do.....	2.06	5.87	.17	10.35
6.....	89.05	.....	.24	1.53	.....do.....	.57	7.12	.14	7.12
7.....	106.85	.....	.40	.79	.....do.....	1.99	6.90	.18	7.10
8.....	124.60	.....	.38	1.24	.....do.....	.43	6.59	.24	5.22
9.....	142.40	.....	.23	.73	.....do.....	.58	5.82	.16	4.90

[a] Heavy infestation.

Two pots of each series were inoculated with pure cultures of *T. basicola*, and young seedlings of the susceptible White Burley variety were transplanted into them. The first test of plant growth failed,

owing, probably in large measure, to poor infestation; and the second test was ruined by a heavy infection of nematodes. All the soils were then sterilized by steam, and two of each series again inoculated, this time by the application of equal quantities of chopped-up, air-dried, heavily infected roots which had been taken in the fall from the tobacco field. The pots were again set to young seedlings of White Burley from steamed soils. The infestation now proved to be good, but complications arose in the limed end especially, owing either to the influence of the lime itself or to the sterilization alone or more likely to the two combined. It seems most probable that the lime was concerned in rendering the phosphates unavailable to the plants, but, as was expected, this injury was probably not a factor in the following experiment. However, this test, which has been designated as experiment I, in Table III, produced some fairly striking results in the infested series.

It was evident during the early growth of the plants that those in the infested soil of highest acidity were making considerably better growth than those at the alkaline end. It also appeared early that the line of demarcation between heavy infection and reduced infection in this series did not lie near the point of neutrality but well into the acid end and so the soil requiring 4.6 tons of lime per acre was as productive of disease as any at the alkaline end. Growth of all the plants was slow as the soil was not very fertile and the light poor. The plants, therefore, were harvested when still relatively far removed from the blossoming stage. They were dried at about 80° C. for several days, and then allowed to come to air-dry weight. The data given under experiment I, Table III, sufficiently illustrates the results obtained. The roots were carefully removed and examined for lesions of the disease, and, as was expected, these were correlated with the growth of the plants. In the soil requiring 9.38 tons lime per acre only a most careful search revealed any *T. basicola* at all. In the next lowest series (7.19 tons per acre), although considerable disease was present, plainly its development was markedly checked, whereas in all the series below this practically no uninfected portions of roots existed.

The crocks were now replanted with Connecticut Havana tobacco, a semi-resistant type. These were allowed to grow for about 50 days before being harvested. The air-dry weights are given under experiment II in Table III. It will be noted that the soil at the alkaline end practically recovered from the injurious properties previously described in the control series. In the infested series it may be noted that the point of effectiveness of the acid reaction in reducing the disease shifted to a somewhat lower degree of acidity, undoubtedly due to the greater resistance of the variety (Pl. 4, I–II). For some unexplainable reason the plant in one of the pots of series 5 and also one in series 7 failed to become as seriously infected as those in the neighboring pots. The increased yields in series 5 and 7, however, are not considered as inter-

fering with the general conclusions to be drawn from the experiment, and the writers have again shown that the highest acidity practically eliminated damage from rootrot, but that heavy infection still occurred in fairly acid soil.

It was believed that the abnormal behavior of the two soils in series 5 and 7 might be due to reduced infestation. All the crocks, therefore, were resterilized and the two of each series reinfested with 100 cc. of a fairly heavy suspension of endoconidia of *T. basicola* in water. This was thoroughly incorporated throughout the 10 kgm. of soil of each crock. Maryland Broadleaf tobacco, a variety almost as susceptible as White Burley, was then transplanted into them soon after inoculation. The results of 65 days of growth are shown in the air-dry weight under experiment III in Table III. A heavy infestation apparently reduced the efficacy of the acid soils to nothing, at least in the presence of a susceptible variety. The disease appeared, in fact, more virulent in the most acid soil.

The soils were now again replanted to Connecticut Havana, the semi-resistant type. The actual amount of infestation was also probably somewhat reduced, as many of the spores originally introduced must have spent themselves, although it is probable that the fungus was living in the soil as a saprophyte. Results similar to those obtained in experiment I were now secured, indicating that partial recovery from infestation had occurred in series 1 and 2.

The question arises as to just what effect soil reaction has upon the occurrence of the disease. High acidity may increase the resistance of the host plant; or it may act deleteriously upon the germination of the spores or the growth of the parasite. If we assume that acidity increases the acidity of the cell sap and, hence, the resistance to disease, as suggested by Comes (*10*) for cereal diseases, we have a working hypothesis which is, however, difficult to establish definitely. It has been shown that *T. basicola* (as do most fungi) grows best on an acid medium (about 1 per cent). Water extracts of the soils from the various series were made which represented approximately the concentration of the soil solutions. Germination tests of endoconidia in these extracts showed better germination in the acid end than in the alkaline end. Other soil extracts tubed with agar showed better growth of *T. basicola* at the acid than at the alkaline end. Although the acidity from the higher acid series was such as would not permit the growth of bacteria, yet tests of this nature probably fall short of resembling the actual acidity in the soil. The results in experiment III show, of course, that spore germination and fungus growth are not completely inhibited by an acidity requiring 9 to 10 tons of lime per acre. The writer is inclined to believe, however, that the beneficial action of soil acidity in reducing infection by *T. basicola* is due to a gradual depressing effect upon the fungus.

FIELD EXPERIMENTS WITH SOIL REACTION

The field plots were located on the Experiment Station farm at Madison on a tobacco field which had grown 10 and possibly 12 successive crops of tobacco, and on a neighboring field which had previously grown only 1 crop of tobacco. The infested field had for three or four years previous to this experiment shown itself to be heavily infested and would grow only half a crop of Connecticut Havana tobacco, while White Burley would make no growth whatever on this soil, especially during relatively cool growing seasons. This soil had had heavy annual applications of barnyard manure and was in a good state of fertility as shown by corn and cereals growing in adjacent plots. The soil reaction at the beginning of the experiments was practically neutral.

A control field across the road on uninfested soil was started for a double purpose. In the first place it made it possible to check up the beneficial or injurious action of the fertilizer and lime applied, aside from infection from disease. In the second place it has been considered that, although the application of acid fertilizer might not remedy the condition in a badly infested field, it might serve to hold down the rate of infestation of new soil to a considerable degree. This soil is equally as fertile as the infested field, but shows a slightly greater degree of natural acidity, being classed as slightly acid according to the Truog color chart. Since it was found in the pot experiments that a very considerable range of reaction was required to make any appreciable difference in amount of infection by *T. basicola*, it was decided to use the more simple though fairly accurate comparative test of Truog (*26*) with lead-acetate paper. The reference to the degree of acidity, therefore, will be based on the standard color chart accompanying the description of this test.

The plots used were one-fortieth acre in size. The applications were made in two different amounts, a heavy application and a light application, also referred to as a full application and a half application, respectively. The original plans of the experiment called for the use of alkaline fertilizers—that is, potassium carbonate, basic slag, and nitrate of soda, with equivalent amounts of sulphate of potash, acid phosphate, and sulphate of ammonia for the acid fertilizers. On account of the apparent impossibility of obtaining all of the alkaline fertilizers, it was decided to use the acid fertilizers and heavy applications of lime to produce the alkaline condition. The rates of applications, in pounds per acre, then, are as follows:

Acid plots.	Full amount.	Half amount.
Sulphate of ammonia	1,200	600
Sulphate of potash	1,200	600
Acid phosphate	2,400	1,200
Alkaline plots.		
Slaked lime	12,000	6,000
Sulphate of ammonia	1,200	600
Sulphate of potash	1,200	600
Acid phosphate	2,400	1,200

The first application was made on June 18, 1917. The applications were made by hand, disked, and harrowed in. The lime was previously allowed to slake in the field. Connecticut Havana tobacco was transplanted on all the plots on June 27. Acidity determinations made one month after the applications showed slightly increased acidity for the acid plots and slightly decreased acidity for the alkaline plots. Determinations unfortunately were not made at the end of the season, but the tests for the following year served to indicate that, although the changes were not great in degree, they were decidedly effective in bringing about a marked change in reaction between the alkaline and acid plots.

In the first year's tests the acid plots in the infested soil showed up decidedly the poorest throughout most of the growing period, while the heavily limed plot was decidedly the best in the series. On the new field the fertilizers both with and without lime gave somewhat better results than the controls. So far as can be judged by the results, the acid fertilizers were not injurious to the crop on this soil although it is possible, of course, that these materials might have had some direct injurious action on the tobacco in the case of the infested soil. Apparently, such a condition did not occur on the uninfested soil, nor on the infested soil when the plots treated in the same manner were limed.

The plots were harvested and cured separately; the yield of cured leaves for 1917 are given in Table IV.

TABLE IV.—*Yield of tobacco on soil with acid fertilizers, with and without lime, 1917–18*

Treatment.	Plot.	Application on 1/10 acre.		Yield of cured leaf on 1/10 acre (pounds).				
				1917.		1918.		
		Acid fertilizers.	Lime.	Infested soil.	Uninfested soil.	Infested soil.[a]	Uninfested soil.[a]	Infested soil.
			Lbs.					
Alkaline....	A...	Sulphate of ammonia, 30 pounds; Sulphate of potash, 30 pounds; Acid phosphate, 60 pounds	300	38.5	44.5	31.3	34.0	31.3
Do.....	B...	Sulphate of ammonia, 15 pounds; Sulphate of potash, 15 pounds; Acid phosphate, 30 pounds	150	26.0	40.0	29.0	39.5	31.0
Control.....	A...	None	None.	24.0	33.5	38.5	39.0	30.0
Do.....	B...	None	None.	22.0	33.0	36.0	.....	28.0
Acid.....	A...	Sulphate of ammonia, 30 pounds; Sulphate of potash, 30 pounds; Acid phosphate, 60 pounds	None.	15.0	44.5	30.5	41.5	30.5
Do.....	B...	Sulphate of ammonia, 15 pounds; Sulphate of potash, 15 pounds; Acid phosphate, 30 pounds	None.	15.5	38.5	29.3	41.5	29.3

[a] 1917 series. These plots received a second application of same amounts in 1918 and had, therefore, the residual effect of the 1917 applications.

The results were sufficiently interesting to warrant repetition the following season (1918) on a slightly increased scale. All the plots were again given an additional application, the same amounts as in 1917 being used. In the infested field six plots were added, these being given

the same treatment as the others, the essential difference being that these did not have the residual effects of the previous season's applications and would therefore be more directly comparable with the plots in 1917.

The applications of lime were made on May 21 and fertilizers applied on June 3. On June 12 all the plots were planted to Connecticut Havana tobacco. On June 21 samples of soil were taken from each of the plots and tested for reaction in the ordinary manner.

In the infested soil the control plots showed very slight acidity. The acid plot of last year (full amount) showed medium to strong acidity; the half-amount plot showed slight acidity. In the same way the plots which had received the acid fertilization for the first time in 1918 showed nearly medium acidity for the full application and slight acidity for the half amount. None of the alkaline plots showed acidity and presumably were considerably below the neutral point, though this could not be shown by the test used. On the uninfested plots the change in acidity due to the application of the fertilizers were not so marked, probably only a slight change having been produced. The limed plots, however, showed no signs of acid reaction.

On June 18 it already appeared that on the full-limed plots the lime was acting injuriously upon the seedlings, both in the uninfested and in the infested soils. This may have been due in part to the fact that the lime was not well air slaked and was, hence, not thoroughly incorporated in the soil. The action of the lime was, therefore, probably toxic and probably vitiated the results, so far as lime was concerned, although the plants appeared to recover later in the season. The outstanding feature of the results in 1918 was again that the plots made acid with heavy applications of fertilizers under field conditions were on the average little or no better than the untreated plots (Table IV). In fact, the untreated plots of the 1917 series were considerably better than the acid-treated plots of 1917 or 1918, although the plots treated with acid fertilizers for the first time in 1918 were slightly better than their controls for this year. There is no question as to the extent of infection on this soil this season, since resistant and susceptible types planted at the same time behaved in the expected manner. In interpreting the results from the field plots it should be recognized that the tests are not exhaustive, and that on account of the complexity of the problem the conclusions drawn may not apply under all conditions. For Wisconsin conditions, however, it appears that the application of acid fertilizers to soils, alkaline or neutral in reaction, will not reduce infection by *T. basicola*.

SOIL TEMPERATURE AS A FACTOR IN ROOTROT

A review of the more important literature concerning the influence of soil temperature on diseases of plants and the importance of such studies has been presented by Jones (*16*). With respect to the influence of this factor on infection and severity of the rootrot of tobacco caused by

T. basicola practically nothing of a definite nature exists. Rather obscure statements that high temperatures favor the disease have been published by Gilbert (*12*), while, on the other hand, Clinton (*8*) states that possibly unusually cold, wet spring weather has something to do with determining whether or not the fungus does much damage. Galloway (*11, p. 174-178*) found that in the greenhouse the disease was apparently more severe on violets on the approach of fall than in summer, indicating a temperature relation. In Italy where very considerable observation has been made on the disease, it is agreed that weather conditions have much to do with its occurrence and severity. That such was the case in Wisconsin was evident during the first season of observation. The recovery of badly infected plants in large areas during the course of only two or three weeks led to the desire to study in more detail the environmental conditions bringing this about. It was at first suspected that the moisture relations were the all-important factor; but in connection with its study, temperature records of the soil under field conditions were taken beginning in the spring of 1915, and continued for the seasons of 1916, 1917, and 1918.

In the fall of 1916, following some interesting results by Tisdale (*25*) on the influence of soil temperature on flaxwilt (caused by *Fusarium lini*), the writers, under the advice and support of Dr. L. R. Jones, undertook to have a large tank (Pl. 2, I) constructed in which soil could be held fairly constant at several different temperatures. This tank has already been described and illustrated in some detail by Jones (*16*). Further detailed description of the mechanical part of the apparatus seems unnecessary, especially in view of the fact that improvements are being gradually made on these tanks which will no doubt necessitate further description of similar apparatus developed in the Department of Plant Pathology of the University of Wisconsin. It should be said, however, that by means of proper insulation of the compartments it has been possible to maintain a fairly constant temperature of water at any selected temperatures between approximately 5° and 40° C. This has been done by the inflow of cold water from the taps in the winter time and by heating the water to the higher temperatures with electric bulbs or with steam. The expense of automatic temperature regulation in a large number of chambers has discouraged the use of such apparatus up to the present time, but personal attention and regulation two and three times every 24 hours, in combination with good insulation, has been found to give results sufficiently accurate for most needs. It was found that although considerable ranges of temperature occurred at the extremes (below 15° and above 30°) the temperatures between 15° and 30° could be held quite constantly within 1 degree.

The soils used were placed in 1-gallon battery jars and set on boards suspended in the water in the tanks. Four jars could be placed in each compartment with displacement of only a relatively small amount of

water. Two jars in each compartment containing sterilized or uninfested soil were used as controls for plant growth alongside two jars containing infested soil. Naturally infested soil from the old tobacco field on the Station farm, previously referred to, was used in most of the experiments. After being given a good application of well-rotted manure, the soil was thoroughly mixed and screened before weighing equal quantities into the jars. The sterilized soil used in the earlier experiments was sterilized by steam to destroy the infestation by *T. basicola.* Considerable difficulty was experienced, however, as a result of the toxic action of the heated soils on plant growth at the lower temperatures, which interfered to some extent with the reliability and uniformity of the data obtained by leaf measurements.

In later experiments the employment of soil steamed two or three weeks previous to being used and allowed to stand in a moist condition at a fairly high room temperature reduced this action to a minimum. In still other tests formalin-sterilized soil was used with equal success, and in the final experiment another uninfested soil was used and artificial innoculation resorted to for the infested series.

The data taken in the earlier experiments were mostly in the form of measurements of leaf area in square inches as determined by a standard chart of various leaf sizes whose areas had previously been determined by the use of a solar planimeter. In later experiments air-dry weight determinations of the stalks and leaves were made.

The determination of the actual amount of disease on the roots is, of course, the final criterion for judgment, and in the last experiments it was found that with care the greater part of the roots could be washed out from the soil, examined for disease, dried, and weighed; these weights are closely correlated with growth aboveground, so that either the area of the leaves, weight of the leaves and stalks, or weight of the roots alone give a good index of the extent of the disease. A preliminary report of the results obtained has been given and an abstract published (*15*).

Eight separate experiments have now been made upon the influence of soil temperature on the extent of the root disease, four determinations being made in the winter and spring of 1917, and four during the fall and winter of 1917–18. The first experiments were made over a range of about 35° C., but, as these were found to be beyond the ranges of normal growth and infection, the later experiments usually included a temperature range of about 15°. Three of the experiments failed more or less to give uniform results; one due to nematode infection at the higher temperatures, another to toxic action of the heated soil, and a third to the accidental use of infected seedlings.

The procedure in each experiment consisted merely in filling the required number of jars with soil; one-half with uninfested and one-half with infested soil. Glass tubes 2½ inches long, were inserted into each jar to permit watering part of the soil at about half its total depth. After

being brought up to about three-fourths saturation, the jars were set in the tanks at the different temperatures and allowed to remain there for three to five days to permit the necessary changes of temperature. One young seedling of tobacco, usually the susceptible White Burley variety, was then transplanted into each jar. Subsequent attention then consisted only in taking the temperature records twice a day, in maintaining the proper temperature, and in watering the plants as required. In the first experiments, when the temperature range was determined, 12 different soil temperatures were run at one time; but in the latter experiments, when a closer approximation of the critical temperature was necessary, only 6 or 7 temperatures were used.

EXPERIMENT I.—Twelve temperatures were used, ranging from 7° to 40° C., and the white burley variety was transplanted into the jars. The plants in the uninfested or sterilized soil series grew best at temperatures of 29° and 31°. Practically no growth occurred below 13°, and again there was poor growth at 40°. From a physiological standpoint it was interesting to note that a marked effect upon the shape of the plants occurred especially at the higher temperatures. While the plants grew low and stocky with broad but rather pointed leaves at the optimum temperature for growth, the plants became tall and spindly, with short and rounded leaves at a temperature of about 36° to 40°.

In the infested soils at temperatures above 26° the plant growth appeared to be almost as good as that in uninfested soil (Pl. 2, I). At the temperatures 23°, 21°, 19°, and 17°, however, a very decided reduction in growth occurred as compared with the uninfested soils at the same temperature.

Upon removal of the roots from the infested soil series it was found that those at temperatures between 23° and 17° were heavily attacked by *T. basicola* and that slight infection occurred at 7°, while at 26° relatively few lesions occurred. The lesions were still less common at 29°, while at 31° only one lesion could be found. At the higher temperatures, 35° and approximately 40°, no signs of Thielavia infection were found.

EXPERIMENT II.—In this experiment the temperature range was 9° to 40° C. The toxic action of the heated soils at temperatures of 17° to 25° became quite marked early in the experiment, and no doubt affected the results. The total leaf area of each plant was determined at four different times during the course of the experiment. The results lack uniformity, however, owing to the toxic action of the heated soils. The infested soils gave the best growth at 35°, with an average of 251 square inches, as compared with 289 square inches for the sterilized soil at the same temperature. The poorest growth was at 19°, where a leaf area of only 19.2 square inches was obtained in the infested soil, as compared with 206.4 square inches in the sterilized soil. While the disease was quite marked at 24.5°, 71 square inches on infested soil as against 205 square inches on sterilized soil, decided indication of reduced severity again appeared at 26°, 169

square inches in infested soil as against 203 square inches on sterile soil. Examination of the roots in the infested series showed a relatively reduced amount of infection at 9° and 13°, heavy infection between 17° and 24.5°, much less infection again at 26° and 29°, and no infection at 31°, 35°, and 40°.

The results of Experiment II are in accord with the results of Experiment I, and apparently show in addition that the optimum temperature for the disease lies around 19° and 21°, although heavy infection still occurs as high as 24.5°.

EXPERIMENT III.—The same soil was used as in Experiments I and II. This soil was now so heavily infested with nematodes at the higher temperatures that the results with *T. basicola* were vitiated, and no data were taken.

EXPERIMENT IV.—New soil from the same infested field was used in this experiment, the proper care being taken to sterilize thoroughly the battery jars before filling them with soil. To reduce the harmful effect of the sterilized soils, the pots, after being filled, were allowed to stand moist for a week at room temperature before being placed in the tanks. In this experiment only seven different temperatures were used, which permitted the use of four jars of infested soil and four controls at each of five temperatures, but only two of each at the extremes. The final results are given in Table V. It may be again noted that the greatest amount of injury from disease occurred at the temperatures from 19° to 22° C., less occurred at 24° to 25°, while at 26° to 27° the injury was much reduced.

TABLE V.—*Influence of soil temperature on development of rootrot*

Temperature.	Average leaf area (in square inches) of duplicates.			
	Experiment III.		Experiment IV.	
	Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.
° *C.*				
13–15	63.3	28.5	71.8	41.7
15–17	106.7	24.9	87.4	13.0
19–20	296.4	19.2	137.6	9.5
21–22	212.3	26.7	304.2	21.4
24–25	205.1	71.1	430.9	197.6
26–27	202.7	168.7	306.1	280.4
30–31	256.4	243.6	339.3	327.1

EXPERIMENT V.—This experiment was largely a failure, owing to the use of plants that apparently were slightly infected by *T. basicola* and also by nematodes. No plants from sterilized soil were available at the time. The results were interesting, however, in that an examination of the roots showed that at the lower temperatures—that is, those favorable to infection—the heaviest infection occurred in the sterilized soil. This

is in line with the frequently observed fact that sterilized soil reinfested is a very favorable medium for the progress of disease. At 31° to 32° C. a trace of infection was found in the sterilized soil, but no infection occurred in the naturally infested soil. Nematode injury was most serious at the higher temperatures.

EXPERIMENT VI.—Six different temperatures ranging between 17° and 32° C. were used. The best growth of the controls in uninfested soil occurred at 31° to 32°, and the poorest at 17° to 18°. The best growth in the infested series was also obtained from 31° to 32°, which was practically equal to that of the controls. Almost equally good growth occurred at 28° to 29°, but at lower temperatures the results were again unfortunately interfered with by the toxic action of sterilized soil, which, though it had been treated for the purpose of reducing the toxicity, had not apparently sufficiently reduced the toxicity. Examination of the roots, however, which were carefully washed out, yielded results in line with the previous experiments.

EXPERIMENT VII.—In this experiment the soil in the uninfested series was sterilized with a 1 to 50 formalin drench three weeks prior to its use, in order to avoid further interference by the toxic action of the steam-sterilized soils. Six temperatures ranging from 17° to 32° C. were again used. This experiment was begun on January 18, 1918, using the White Burley variety in the same soil as previously used, and concluded on February 26. Marked differences in growth on the uninfested and infested soils were already noticeable on February 5; the plants in the sterilized soil 20° to 21° and 23° to 24° were twice as large as those in the infested soil; whereas the plants in the infested and uninfested soil at 31° to 32° were practically equal in size. The final results are shown in Table VI A, in terms of air-dry weight of the stalks, leaves, and roots in the infested and uninfested series, together with the amount of infection on the roots. It may be seen that the best temperature for growth in this case was apparently 28° to 29° for the above-ground portions of the plant, but that the best root development took place in the cooler soil at 23° to 24°. In the infested soil a gradual increase in growth from the lowest to the highest temperature is evident. (Pl. 2, II–III.) Practically the same is true for root development. (Pl. 2, IV.)

EXPERIMENT VIII.—In this experiment an ordinary greenhouse soil mixture, free from *T. basicola* was used. No sterilization, therefore, was used, and infestation with *T. basicola* was produced by thoroughly incorporating a heavy spore suspension of endoconidia of the fungus from young cultures on agar. The experiment was now run as before, except that the Connecticut Havana variety, which is relatively much more resistant to *T. basicola* than the White Burley, was used. The young seedlings were transplanted on March 6, 1918. On March 17 the plants in the inoculated pots already showed signs of heavy infection at the lower temperatures. On days of high transpiration the

plants in the inoculated series wilted first at from 22° to 23° C., but no wilting occurred at 31° to 32° or in the inoculated soil. On April 15 the experiment was terminated, the stalks and roots were cut and dried, and the roots washed out as carefully as possible and dried. The air-dry weights are shown in Table VI B.

TABLE VI.—*Influence of soil temperature on the rootrot of tobacco*

A. WHITE BURLEY VARIETY, NORMALLY INFESTED SOIL

Series.	Temperature of soil.	Average air-dry weight per plant.				Amount of infection in infested soil.
		Stalk and leaves.		Roots.		
		Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.	
	°C.	Gm.	Gm.	Gm.	Gm.	
1.....	17–18	3.6	0.35	0.32	0.03	Heavy; roots all black.
2.....	20–21	5.4	1.80	.66	.07	Do.
3.....	23–24	7.1	1.70	.86	.10	Not quite as heavy as in series 1 and 2.
4.....	25–26	6.9	2.75	.79	.17	Considerable, but much less than in series 3.
5.....	28–29	7.6	3.8	.70	.16	Slight infection.
6.....	31–32	5.9	5.7	.25	.28	No definite signs of disease.

B. CONNECTICUT HAVANA VARIETY, ARTIFICIALLY INFESTED SOIL

1.....	12–13	1.8	0.53	0.26	0.16	Heavy; very few white roots.
2.....	17–18	7.9	.65	.70	.15	Heavy; about same as in series 1.
3.....	22–23	9.1	1.15	1.05	.19	Heavy but less than in series 1 and 2.
4.....	26–27	10.6	3.75	1.13	.38	Much less than in series 3.
5.....	28–29	10.8	8.10	1.13	.90	Very slight.
6.....	31–32	10.5	7.20	.75	.60	No sign of infection.

The largest yield of the above-ground portions of the plants in the uninoculated series occurred at 28° to 29°, but was only slightly larger than at 26° to 27° or 31° to 32°. The largest root development occurred at 26° to 27° and 28° to 29°, with a decided falling off at 31° to 32°. In the inoculated soil the largest yield of the above-ground parts was at 28° to 29°, with some falling off at 31° to 32°, though not due to infection. It should be noted here that the greater reduction in yield is at 17° to 18°, the disease apparently less marked at 12° to 13° and at 22° to 23°. Practically this same relation holds for the roots. This, together with other experiments, seems to indicate with considerable certainty that the amount of infection and severity of the rootrot are most marked at temperatures ranging between 17° to 23° C. At temperatures below about 15° the extent of the disease is reduced, but this temperature also is too low to permit any growth of tobacco, and consequently is of little practical importance. On the

other hand, at temperatures of 26° and above, the amount of infection and the extent of the injury done are gradually reduced until at about 30° no appreciable injury results, and at at 31° to 32° it is permissible to say that practically no infection whatever occurs.

The results having shown that the rootrot can be practically controlled by high soil temperatures, which at the same time are favorable for the growth of tobacco, the question naturally arises as to how far a plant may recover from serious root infection, provided a change of soil temperature from one favorable to disease to one unfavorable to disease is brought about. Eight White Burley plants which had been planted to the infested tobacco field in June but which had made no appreciable growth during the entire season in the field were taken up late in September with their adhering soil and transplanted into the jars with the infested soil. Four of these were then set in the temperature tanks at a low temperature (20° to 21°) and four at a high temperature (30° to 31°). After remaining at these temperatures for a month the roots were dug out as carefully as possible, and the results are illustrated in Plate 3. The plants had almost no roots when placed in the tank, and one must marvel at the wonderful persistence of tobacco plants in maintaining themselves with an almost complete lack of root system. At the higher temperature, however, new roots were forced out through the blackened bases of the stalks and remained uniformly clean, white, and free from disease. This experiment was repeated with even more striking results by moving jars with badly diseased plants from the low temperatures to the high temperatures in the tanks. In the space of three or four days the plants seemed to have taken on renewed vigor and growth. These experiments seem to prove beyond doubt that similar conditions may happen in the field under practical conditions, and that the phenomena of recovery of a badly diseased crop, so frequently noted in the field within a short period of time, is no doubt due in large measure to natural changes in temperature relations of the soil.

SOIL TEMPERATURES IN THE FIELD

It now remains to ascertain how far the soil temperatures occurring under normal conditions in the field may influence the actual amount of infection and damage from rootrot. It is necessary, therefore, to determine the actual soil temperatures occurring during the growing season in order that a knowledge may be obtained of the change occurring at different times in the same season and during different seasons taken as a whole. Unfortunately not a great many reliable data upon soil temperatures for summer months in various sections of the country exist. Such as do exist, however, may have a bearing upon future studies of the influence of soil temperatures upon the occurrence of disease. It is to be expected that soil temperatures have a fairly con-

stant correlation with air temperatures, and it is highly probable that a fairly constant ratio may be calculated which will enable the extensive data on air temperatures to be used in considering relations of soil temperatures to disease.

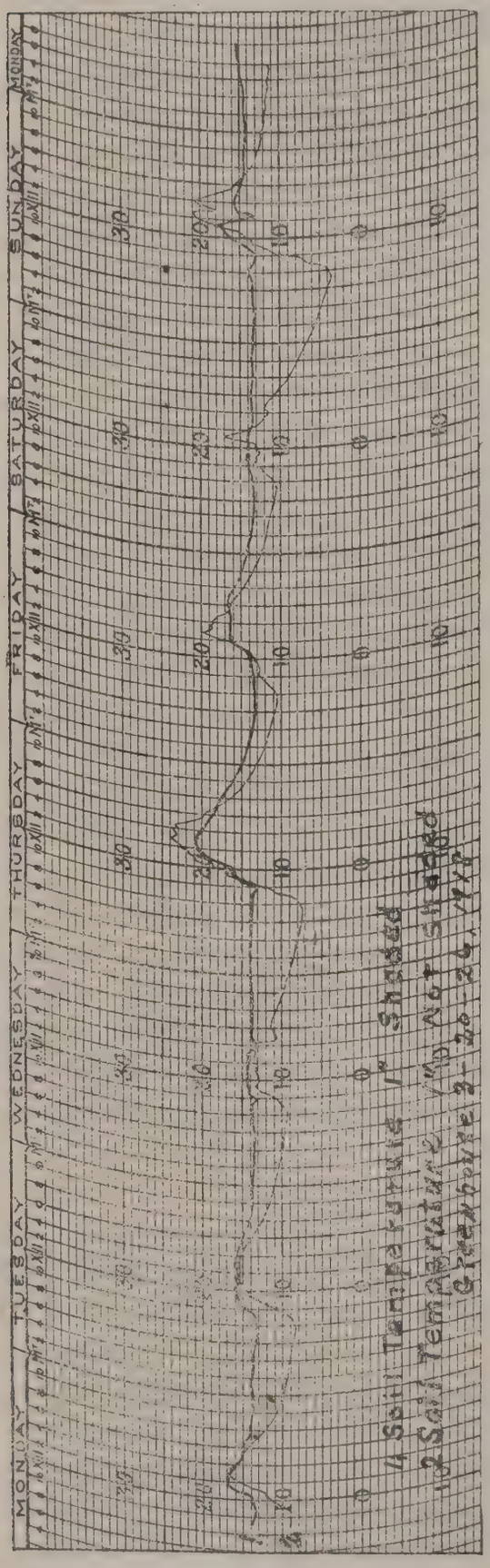

FIG. 1.—Soil-thermograph records showing the influence on soil temperature of the shading of soil by growing tobacco.

The data taken in connection with the studies presented in this paper were started in the spring of 1915. For this purpose electrical resistance thermometers were used. These were buried in the soil in tobacco fields at the Station farm at depths of 2, 4, and 8 inches. Some of the thermometers were buried in such a way that they would record the temperature of soil becoming gradually shaded by the growing tobacco, while with others the soil was exposed continuously to the full sunshine. The latter temperatures are the ones upon which conclusions were drawn, since in a badly infested field shading of soil would be relatively small, owing to the poor growth of the crop. On the other hand, where very heavy infestation does not occur or a relatively resistant variety is used, the relative importance of shading must be considered (fig. 1).

The temperature readings were taken with duplicate thermometers each day at 1 p. m. This hour was selected as it was the most convenient time of the day to take the readings. No great importance, however, can be attached to the time of taking daily readings, on account of the great daily variation which occurs especially near the surface of the soil. It would be most desirable to record the minimum and maximum temperature for each day, but in using electrical thermometers this would

entail too many readings. Temperature readings taken at 7 a. m. and at 1 and 5 p. m. for one week (July 6 to 13, 1916) showed that the temperature was anywhere from 1 to 5 degrees lower at 7 a. m. than at 1 p. m. and to average slightly higher at 5 p. m. than at 1 p. m., indicating that the maximum perhaps was reached at some time between 1 and 5 p. m., and on clear days at about 3 p. m. A recording soil thermograph was also used at a depth of 4 inches in 1917. From these records it may be noted that the highest temperature usually occurred about 4 p. m. (fig. 2).

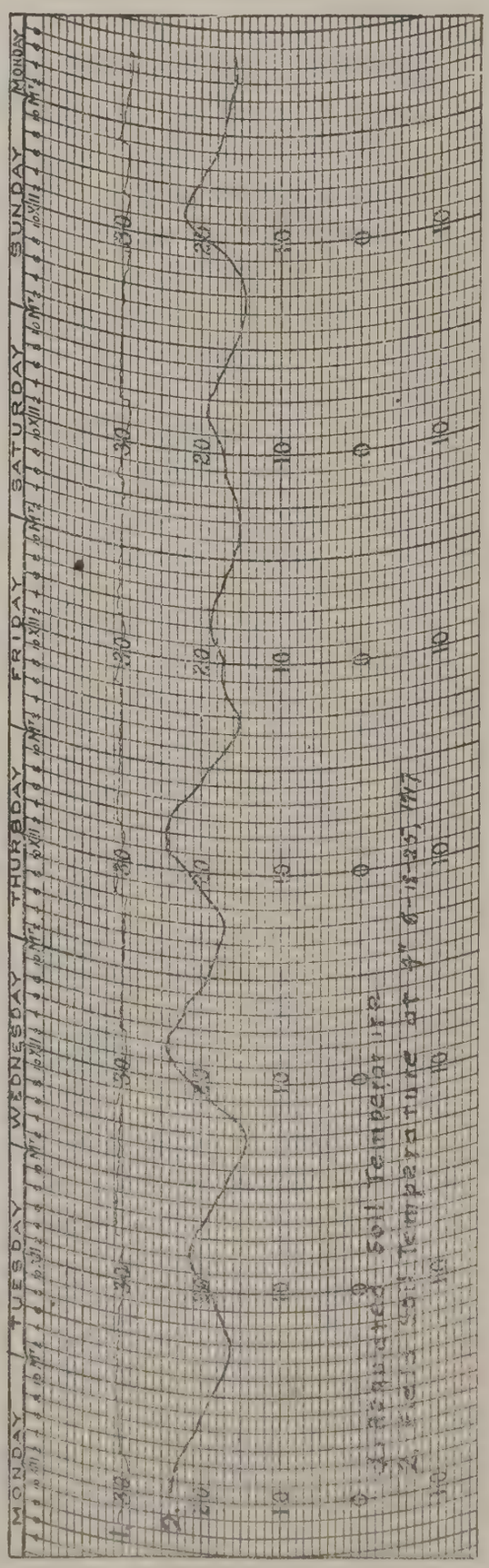

FIG. 2.—Soil-thermograph records giving comparison of a typical record of regulated soil temperature in tanks with a typical record from the field at a depth of 4 inches.

A more important consideration, however, is the general rise or fall of temperature during extended periods of a week or more, or the general trend of the temperature for one season as compared with another.

In the northerly latitudes the growing period of tobacco, practically speaking, lies within the months June, July, and August. Although much tobacco remains in the field during the month of September, practically all the growth must be made before that time. July undoubtedly is the critical month in which most of the growth should be manifested, although if growth is retarded until August, and a warm fall follows, with absence of frost until late into September, a marketable crop may often be produced. In the northern districts nearly all tobacco is planted in June. From the standpoint of temperature this is the most favorable month for the rootrot. Heavy infestation in June followed by a warm July, however,

may overcome the disease. If the warm period is delayed until late July or August, recovery may still be made and a late crop of good yield produced, provided the balance has not swung to the other extreme—that is, forced maturity.

No condition is more commonly seen in infested tobacco fields than that of plants budded out and ready for topping two to three weeks before the normal date when the plants have obtained only one-fourth to one-half their normal growth. This is a direct result of the starvation of the plants caused by disease. A drouth may bring on the same condition. The plants then must be topped when this stage is reached, and although much spread of leaf may subsequently occur, owing to the arrival of more favorable conditions for growth, yet the yield is almost certain to be light.

Several years of practical observation of infested fields have shown that heavy infection almost always occurs in June. Every tobacco grower of experience, at least in Wisconsin, can cite cases where during the first two or three weeks after planting the crop prospects have been excellent, followed by a like period of uncertainty, when the condition of the crop has apparently made no progress or has gone slightly backward, and finally, for no apparent reason, where the crop has taken on a new lease of life, or, on the contrary, has remained to the end more or less of a failure. In Wisconsin a large percentage of poor crops in the years 1913, 1915, and 1917 was due either to poor yield or delayed maturity directly traceable to the rootrot. In the years 1914 and 1916 fairly good yields were obtained, and not much root disease occurred even on infested soils.

It is believed that an examination of the summarized soil temperature records for these years in Table VII, or a glance at the temperature curves in Plates 6–8, will furnish in a large measure an explanation for the results obtained with tobacco in 1915, 1916, 1917, and 1918. The year 1915 was an especially cold season; according to weather bureau records at Madison it was the coldest on record, and also a comparatively wet one. The studies of the writers on the influence of soil moisture, however, have now convinced them that its importance as a controlling factor under field conditions is small as compared with temperature. In 1915 the loss from the rootrot of tobacco was estimated at from $10,000,000 to $20,000,000 in the United States alone. The year 1918 showed very poor prospects of a good crop for a period of several weeks in July and early August. In the latter half of August, however, the Wisconsin crop made a remarkable growth even in the most heavily infested fields; this growth was unquestionably a direct result of the increased soil temperatures during this month.

TABLE VII.—*Average monthly and seasonal soil temperatures for tobacco-growing periods, 1915–1918, at different depths of soil*

Season.	Depth of soil.	Temperatures during month of—			Average for growing period.
		June.	July.	August.	
	Inches.	°C.	°C.	°C.	°C.
1915	2	20.9	20.9	19.0	20.3
	4	20.4	20.6	19.1	20.0
	8	18.0	19.2	18.3	18.5
1916	2	27.6	31.8	23.8	27.7
	4	23.0	27.8	22.7	24.5
	8	18.6	24.3	22.4	21.8
1917	2	21.7	28.2	27.6	25.8
	4	18.7	24.5	24.8	22.7
	8	16.3	21.6	21.5	19.8
1918	2	23.8	27.0	29.0	26.6
	4	21.0	23.6	25.4	23.0
	8	17.9	20.5	21.8	20.1

The practical bearing of this problem is manifold. In so far as seasonal temperatures can be judged and predicted, crop prospects on infested soils can be predicted, and in the northern tobacco-growing sections the infested soils usually constitute anywhere from one-half to three-fourths of the acreage grown. In so far as "warm" soils can be selected—that is, sandy, dark soils with good drainage and a southerly exposure—in preference to "cold" soils, the possible extent of the damage from disease has been reduced. If the crop is planted early on infested soils, heavy infection is more likely to occur in the early stages of plant growth, and the plants will find it more difficult to recover. One of the most common beliefs of the Wisconsin grower, based on observations of several years, is that early planting means plants budding out in July, and an early, light-weight crop. From a purely physiological standpoint there could be only one possible explanation for such behavior of early-set tobacco, namely, the more or less common occurrence of a drouth in July. The inadequacy of such an explanation, however, is shown by the following observations: The vigor of growth of corn and other cultivated crops has remained practically unchecked during many of these frequently recurring so-called drouths in July; likewise, the growth of ordinary tobacco on new soil of a neighboring farm or of a resistant variety in the adjoining row on an infested field has not been greatly interfered with by these weather conditions; finally, exceptionally poor crops of tobacco were grown in Dane County, Wis., in the years 1913 and 1915, whereas the July rainfall was 8.47 inches in 1913 and 5.04 inches in 1915, both greatly in excess of the normal.

It would seem that some value could be attached to late planting on soil infested by *T. basicola*, in view of the low temperatures in June.

On the other hand, the practical application of such a recommendation is doubtful on account of the variations in seasons as to temperature and general growing conditions. In general the farmers must transplant to the fields when the seedlings are of proper size, a matter which usually can not be predetermined very effectively for more than two or three weeks. With steam-sterilized seed beds closer approximations can be made, and seed may be sown two to three weeks later than normally, with fair certainty of obtaining plants by June 20 to 30. Planting later than July 1, however, is no more certain of giving satisfactory final results than early planting.

During the season of 1917 a planting experiment was carried out, with the hope of getting some data on this subject. Seedlings were transplanted at intervals of one week from June 11 to July 23 on infested and uninfested soil. Unfortunately, the White Burley variety was used on heavily infested soil, and the season being relatively cool throughout, no appreciable difference in yield occurred on the infested soil. On the uninfested soil, however, the advantage was all with the early-set tobacco; a gradual decrease in size and value occurred in the later plantings. A wide range of observation has convinced us that, other conditions being alike, early-planted tobacco on uninfested soil usually is considerably safer than late-set tobacco on either infested or uninfested soil.

Before leaving this subject another point of more scientific interest should be considered: Why are tobacco roots most seriously attacked by *T. basicola* at from 17° to 23° C. and practically not at all at a temperature of 30° C.? Several hypotheses may be formulated. The simplest explanation would be that the resistance of the roots to the parasite is modified at different temperatures, high susceptibility occurring from 17° to 23° and practical immunity at 30°. At first sight a tenable theory seemed to be that the increased vigor of root formation at higher temperatures sufficed to overcome the destructive effects of the disease. On the other hand, the action of temperature variation may be regarded as modifying the ability of the fungus to grow in the soil or to attack the host. On the basis of some preliminary experimental results, the latter theory seems to be the most probable.

It should be noted, however, that the behavior of the parasite in culture does not correspond entirely with its behavior on the host as regards temperature relations. Gilbert (*12*) found the following critical temperatures for growth: Minimum 7° to 8° C., optimum 30°, maximum 34° to 37°. The determinations of the writers have given figures very much the same as these. The temperature most favorable for infection does not therefore agree with the optimum for growth in culture. On the other hand, the optimum growth in culture is obtained at 29° to 30°, where the organism is apparently ineffective as a parasite. It is not possible, therefore, to draw any decisive conclusions as to the behavior of the fungus as a parasite from its behavior in artificial culture

media. At temperatures of only 3 or 4 degrees above the optimum in culture, however, the fungus, though making some growth, behaves quite normally, and it is not difficult to conceive of no infection or growth occurring on the host at temperatures above 30°. The results are probably in line with the relation of temperature to infection with other parasites, where it is known that the fundamental factor concerned is that of spore germination.

The following brief description of some experiments may be of interest: The roots of tobacco plants in 7-inch pots were forced to grow out through the perforation in the bottom of the pots by setting them in battery jars partly filled with water. After the roots had made a good start, the jars containing the plants were set in the temperature tanks, at high and low temperatures, 31° to 32° C. and 17° to 18°, respectively. They were allowed to remain there for a week to 10 days. During this time many fresh roots were formed. Two jars at each temperature were now removed to a temperature of 23° to 24°, and young endoconidia of *T. basicola* introduced into the water surrounding the roots. Other plants remaining at 31° to 32° were also inoculated in a similar manner. Good visible infection occurred in 3 to 4 days in all plants at 23° to 24°. No difference was observed at this time or later in the roots which had formed, either at a high or at a low temperature. No infection occurred at 31° to 32° after 8 to 10 days, but infection did occur when the pots were removed to a lower temperature without reinoculation. This showed that the fungus had not been destroyed.

This test at least demonstrated that the increased growth of the host at higher temperatures is not due to the overcoming of the effects of the disease by increased root development, but is due to the inability of the fungus to infect the host. It also tends to show that any resistance or susceptibility at high or low temperatures which the roots have developed is rapidly lost, since infection must have occurred within 24 to 36 hours after changing from the extremes to the medium temperature. There is room for a great deal of investigation, however, upon the intimate environmental relations of host and parasite in this disease, and it is expected that this subject will be treated in more detail in another paper.

INFLUENCE OF ORGANIC MATTER IN THE SOIL

The content of organic matter and humus in the soil has been ascribed by most investigators of tobacco rootrot as being a very influential factor in determining the amount of disease. Practically all the writers agree that the addition to the soil of vegetable matter in the form of green manures or barnyard manures increases the extent of the disease. Massee (*19*) has gone so far as to state that the disease can not occur at all in the total absence of organic matter, since he believes the fungus must gain some stimulus while living as a saprophyte before being able to penetrate the host. Nearly all of these conclusions, however, have

been based on observation rather than on experimental data. The question is an important one from a practical standpoint. Will the selection of soils low in organic matter or avoidance of the use of green or barnyard manures materially aid in reducing the disease?

This is one of the most difficult problems to subject to experimental test in such a way that wholly reliable conclusions can be drawn. It illustrates equally well the fallacy of drawing far-reaching conclusions from mere field observation. It is evident that the organic matter of the soil has profound influence upon a large number of other factors such as water-holding capacity, food supply, temperature, reaction, texture, aeration, and saprophytic growth of microorganisms in the soil. To eliminate all these factors, even in the most carefully controlled experiments, is impossible. To judge of their relative importance in the results obtained in an experiment, however, on the basis of the behavior of such factors from other experimental evidence, is quite likely to yield fairly reliable results.

TABLE VIII.—*Influence of amount of organic matter in soil on rootrot of tobacco*

Series.	Relative percentage of leaf mold to ground quartz by weight.	Experiment I.				Experiment II.			
		Loss on ignition.	Average air-dry weight per plant.		Ratio.	Loss on ignition.	Average air-dry weight per plant.		Ratio.
			Uninfested soil.	Infested soil.			Uninfested soil.	Infested soil.	
		Per cent.	*Gm.*	*Gm.*		*Per cent.*	*Gm.*	*Gm.*	
1	0	0.22	0.88	0.014	1 : 63	0.24	3.35	0.25	1 : 13
2	10	1.65	5.07	.020	1 : 253	1.05	3.35	.42	1 : 8
3	20	.....	.....	.....	.....	2.85	4.90	.60	1 : 8
4	40	5.85	5.93	.052	1 : 114	7.45	5.60	.32	1 : 17
5	60	9.67	7.11	.107	1 : 66	12.7	5.55	.29	1 : 18
6	80	24.1	6.17	.179	1 : 35	23.5	7.00	1.85	1 : 4
7	100	40.8	8.01	.156	1 : 51	52.7	7.27	.70	1 : 10
8	[a] 100	40.8	7.19	.072	1 : 99	.....	.....	.....	.....

[a] Leaf mold heated to 110° C. before infestation.

An attempt was therefore made to arrive at such conclusions by studying the behavior of the disease in the purest ground quartz sand available and also in pure leaf mold, together with mixtures of the two in various proportions (Table VIII). The chief difficulty met with at once in such a combination is to obtain an approximately uniform supply of plant food in these various media. The leaf mold was found to contain sufficient plant food to support normal growth, though after the third crop the plants showed potash hunger. To the pure quartz cultures a nutrient solution sufficient for plant growth was added, and decreasing amounts were added to the various mixtures of sand and leaf mold with a rough estimate of the amount of nutrient salts required. Two pots of each series were then inoculated with *T. basicola* and two

left uninoculated as controls. All were then transplanted with the White Burley variety from sterilized soil. It became evident at once that on uninfested soil the rate of growth of the plants in the extremes of the series was markedly different; the leaf mold was much more favorable for growth than the sand with nutrient solution. Several tests were run on these pots, and also on another series made up in a similar manner (Experiment II, Table VIII). Most of the data concerning them exists as notes and estimates of relative growth during the progress of the experiment. Much reliance can not be placed on the weights, owing to the large variation in fertility, although the ratios given of the growth on infested to that on uninfested soil indicate the general trend of the results.

The experiments, of which there were a considerable number, can not profitably be discussed here in detail. Owing to the variation in results obtained in growth, much reliance was placed on estimates of actual infection on the roots themselves, estimates difficult to express in figures. After summarizing the results of all the tests run (nine in number), it can be stated with considerable confidence that the importance of organic matter in the soil is relatively small, so far as infection and severity of the disease are concerned. It seems, however, that heavy infestation is more rapid, and is more likely to be maintained through unfavorable periods for the parasites in soils high in organic matter rather than in those low in organic matter. Given a uniformly heavy inoculation of the soil with endoconidia of *T. basicola*, the rate and severity of infection is apparently practically the same in pure sand as in the pure leaf mold. Massee's conclusion (*19*) that *T. basicola* is a weak parasite and unable to infect the host except in the presence of organic matter seems entirely unwarranted. This has been further shown by infections obtained from spore suspensions in pure water or spores alone placed directly upon roots grown in a moist atmosphere. After the lapse of a considerable period of time from the date of inoculation, however, it seems certain that *T. basicola* becomes more firmly established in pure leaf mold than it does in pure sand, although this is apparently a difference of amount of infestation and not one of virulence (Pl. 4, V–VI).

With regard to the various mixtures of sand and organic matter, the conclusion seems justified that, so far as infection following inoculation is concerned, it takes place with equal ease in all (Pl. 4, III–IV). The development of infestation of the soil, however, has not given quite the expected results. Doubling or tripling the content of organic matter apparently has not increased infestation, and in some cases increasing the ratio up to 80 parts of leaf mold seemed actually to reduce it. The results, however, have not been sufficiently uniform in this respect to warrant a final conclusion, and it is not certain that factors other than the organic matter do not play a part here. Nevertheless, the fact that increasing the organic content of the soil, two, four, six, and eight times,

on the basis of percentage loss on ignition, has not consistently increased the amount of disease seems to warrant the conclusion that the growers have little to fear in the way of increased infestation of the soil as a result of plowing under green manures or applying 10, 20, or 40 tons of manure to the acre.

INFLUENCE OF THE CLAY AND SAND CONTENT OF THE SOIL

The value of sand or sandy soils in reducing the severity of the rootrot and its increased severity in clay soils has been especially suggested by Benincasa (*2*) and Gilbert (*12*).

A pot experiment with pure quartz sand and with the purest clay obtainable was carried out with the hope of throwing more light upon this subject. The only factor which it is desirable to vary in such an experiment is the size of the soil particles. Although this is not practicable, the relative proportion of sand and clay particles no doubt resembles soil conditions equally well. Superior red clay was obtained from the substation of the State experiment station located at Ashland, Wis. This is a very "heavy" pure clay soil containing very little organic matter and is low in fertility. The sand used was a medium to coarse ground quartz. The mixtures of sand and clay made were those shown in Table IX.

TABLE IX.—*Influence of relative amount of clay and sand on rootrot of tobacco*

Series.	Mixture.		Average air-dry weight (in grams) of plants.			
			Experiment IV.		Experiment V.	
	Sand.	Clay.	Uninfested soil	Infested soil.	Uninfested soil.	Infested soil.
1	All	None	1.60	0.40	0.75	0.26
2	Three-fourths	One-fourth	1.0	.25	.61	.20
3	One-half	One-half	.90	.20	.52	.16
4	One-fourth	Three-fourths	.75	.25	.50	.25
5	None	All	1.10	.15	.30	.13

The experiment in this case was confronted with practically the same difficulties and complications as occurred in the tests with organic matter. The clay soil alone, or in mixtures with sand, seemed to have an "injurious" action upon the growth of tobacco which was not remediable with nutrient solutions applied. The yield, therefore, was low in all cases. Two pots of each series were inoculated with equal volumes of spore suspension from cultures of *T. basicola* on agar, which were thoroughly mixed with the soils. White Burley tobacco was then transplanted into them. The first experiment was started November 18, 1916. The result of this experiment was not recorded by weight, but some of the crocks were photographed (Pl. 5, I), and serve to illustrate the results obtained. The conclusion drawn from this experiment was that

sand was considerably more favorable to infection of roots with *T. basicola* than was clay.

Another test with the Maryland broadleaf variety, started on October 13, 1917, gave practically the same results; root examination showed the greatest infection with sand, less with an admixture of one fourth clay, and almost none with one half clay, and still less with larger amounts of clay. However, in one pot heavy infection occurred at the base of one plant, and the results were interfered with somewhat by nematode injury.

All the soils were then resterilized with steam and two pots of each series heavily infested with a suspension of young endoconidia of *T. basicola* from agar slants. White Burley was again replanted into all the crocks. The results obtained in this case differed somewhat from the preceding, owing most probably to heavier infestation. Infection seemed to occur most rapidly and severely in the one half and three-fourths clay mixtures, but in a few days the plants in the infested series were practically identical in appearance, and after about four weeks, nearly all were killed.

These were now removed, and a more resistant variety, Connecticut Havana, transplanted into the pots. After 18 days all these also were practically equally diseased in the infested series, and were not quite half the size of those in the uninfested series. After about six weeks' growth these plants were cut and the air dry weights determined as shown in Table IX, Experiment IV. The pots were again planted to tobacco with similar results (Table IX, Experiment V). The results of the last experiments seem to indicate that in the presence of heavy infestation of the soil very little difference exists between clay and sand mixtures in the severity of infection of tobacco by *T. basicola*.

The results obtained in the first experiments are believed to be due to the fact that the parasite found clay soils unfavorable for growth and multiplication, and especially for penetration of mycelium as compared with the sand, and therefore less actual infection occurred. On the other hand, with heavy infestation sufficient spores were in close proximity to the roots to produce good infection at once.

With respect to the persistence of *T. basicola* in soil, and its gradually increasing infestation in spite of unfavorable conditions, it is believed that clay soils may eventually be more injurious than sandy soils, but the results seem to justify the conclusion that from the standpoint of texture alone, the selection of loose sandy soils, or the use of clay soils does not necessarily predetermine to any important degree what the injury from *T. basicola* will be. It should be added that clay soils draining more poorly and warming up more slowly, undoubtedly may be considerably more harmful than sandy soils, because of the influence of saturated soils and low temperature upon the severity of the rootrot. It is also believed that the tendency of the clay soils toward greater compactness may also favor somewhat the occurrence of the disease.

INFLUENCE OF SOIL FERTILITY

In the case of soil-infesting parasites which cause the loss of large portions of the root system in such a way that it can no longer function normally for the benefit of the plant, it seemed probable that the quantity of available plant food would influence growth in infested soil. It may be supposed, for instance, that, if in a soil low in fertility one-half of the roots are destroyed by disease, doubling the quantity of available plant food would materially reduce the actual amount of damage done in yield of crop. This is in accord with the conclusions of Briggs (*3*) on this subject. On the other hand, there are the views expressed by many pathologists with respect to various diseases, and also by Gilbert (*12*) and others for the Thielavia rootrot, that an increase of fertilizers, especially those of nitrogenous nature, renders the plant more susceptible to attack. Aside from these theoretical conclusions, we are confronted with the facts that the tobacco rootrot as it occurs in the field is not confined especially to soils in a low or high state of fertility, and that the application of fertilizers, whether as barn manure or commercial fertilizer, seems to have no marked effect upon the relative amount of disease, or on the growth of the plants in infested soil in seasons favorable to rootrot. These conclusions are based on four years of fertilizer plot experiments carried on at Edgerton, Wis., during the years 1910 to 1913, inclusive, the detailed results of which can not be given here. These experiments were planned to determine if it is possible to remedy the "worn-out" or "deteriorated" condition of old tobacco soils by the use of fertilizers of various sorts, although the full significance of *T. basicola* as the cause of this condition was not recognized at the time the experiments were started, and it was not until after three years of failure to obtain any marked results with fertilizer treatments on a wide variety of old soils, coupled with highly beneficial results on soil new to tobacco that the importance of *T. basicola* in crop production was fully realized.

An experiment to determine more carefully the effect of plant food applied in the form of pure salts on the severity of the rootrot was carried out in pot tests in the greenhouse during the winter of 1917–18. Twenty-four 2-gallon crocks with a drainage perforation at the base were each filled with 10 kgm. of soil infested with *T. basicola* from the old tobacco field on the station farm. Twelve of these were now sterilized by steam to destroy the fungus. The cultures were divided into six series of four pots each, two containing infested and two uninfested soil. A complete fertilizer was made up from chemically pure salts according to a formula used for nutrient water cultures, as follows:

	Gm.
Calcium nitrate	[illegible]
Potassium chlorid	[illegible]
Magnesium sulphate	10
Tribasic potassium sulphate	10

This fertilizer was added to each pot in each series and thoroughly mixed with the soil in the amounts shown in Table X; the application ranged from one which was considered only light, to one which was so heavy as to decrease materially the yield. In these pots three successive crops were grown; the first being the susceptible White Burley; the second the semi resistant Connecticut Havana; and the third the susceptible Maryland Broadleaf variety. The average air-dry weights for the plants in the infested and the uninfested soil for the three crops are given in Table X:

TABLE X.—*Influence of fertilizer on rootrot of tobacco*

Series.	Fertilizer added.	Average air-dry weight (gm.) per plant.					
		First crop (White Burley).		Second crop (Connecticut Havana).		Third crop (Maryland Broadleaf).	
		Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.
	Gm.						
1		7.65	0.95	7.97	4.62	5.60	0.52
2	3.5	7.63	2.25	12.05	3.87	5.25	.60
3	7.0	8.90	.57	11.75	3.60	5.85	.45
4	14.0	11.57	.17	12.17	3.75	9.40	.40
5	28.0	8.15	.48	11.00	1.50	16.80	.31
6	56.0	3.80	.18	6.10	.59	6.42	.15

It may be observed at once that the uninfested soil responded to the fertilizer treatment; the maximum yield for the first and second crops was in the pots which received 14 gm. of fertilizer. Doubling the amount of salt, however, decreased the yield, and quadrupling it acted very injuriously, presumably owing to increased concentration of the soil solution. Very poor growth was made on the infested soil in all cases (Pl. 5, II-III). In the first experiment, though, the lowest application of fertilizer apparently increased the growth, followed, however, by a decrease at higher applications. The most striking fact was that the most beneficial application on the uninfested soil showed no signs of such beneficial action on the infested soil.

The results obtained with a more resistant variety as a second crop are believed to be more representative. In this case there is a gradual decrease in yield with the application of the nutrient salts on infested soil; no increase from the application of 3.5 gm. of fertilizer occurred as in the first crop grown. This influence of a light application of fertilizer to infested soils is apparently in need of further investigation on a wide range of soils and with different varieties. From a practical standpoint it seems safe to conclude that fertilizer as such is wasted when applied for tobacco on soils badly infested with *T. basicola*, and that it may, in fact, do more harm than good.

Theoretically we are concerned with the reasons for the injurious action of nutrient salts on the growth of tobacco in infested soil, or more directly, the increased severity of the disease in the presence of increased supply of nutrient salts. A root system reduced by *T. basicola* evidently is not able to increase its functions in the presence of increased fertility, in substitution for the lost roots; but it still seems as though this should be possible, provided other factors do not interfere.

There are at least three plausible explanations for the observed behavior: (1) the increased concentration of the soil solution may favor fungus growth; (2) increased food supply may favor increased susceptibility to disease; (3) the reduced root system in the presence of increased concentration of soil solution may not have been able to furnish a sufficient supply of water to the plants. The first explanation seems most plausible and yet seemingly can not wholly account for the results obtained. The second, that of increased susceptibility, seems least plausible, since no one has yet satisfactorily shown that actual susceptibility to disease is increased by increased fertilization. There is some reason to suppose that the third hypothesis is a factor. In the uninfested soil a good illustration of the injurious action of high concentration of soil solution on plant growth is found. This is explained as an osmotic relation, the entrance of water to the plant being reduced, owing to the high concentration of the soil solution. It seems probable, therefore, that in the presence of a greatly reduced root system this condition would be exaggerated with a resultant reduced growth. The water-relation theory is strengthened by the observations on the relative time and extent of wilting of plants on days favoring high transpiration. Wilting of tobacco plants during periods of high transpiration on infested soils and rapid recovery is quite common and indicates a delicate water relation existing between the plant and the soil.

INFLUENCE OF COMPACTNESS OF SOIL

Field observations have seemed to indicate that in many instances where the soil in infested fields is hard or compact, owing to poor preparation of the soil or to other causes, the damage from *T. basicola* is more marked than in loose soils. In fact, many farmers have been found who have attributed poor yields to compact soils alone, when, as a matter of fact, rootrot was undoubtedly the primary cause. It is, however, very difficult to say just how much injury is due directly to the hard compact or baked condition of the soil and how much is due to its influence on the progress of the rootrot when present.

A simple experiment to determine this point was carried out. The naturally infested soil from the field was carefully screened and mixed in a relatively moist condition. A 6-inch clay pot was filled with the soil in as loose a condition as possible. This held 2,000 gm. Another pot was then filled with the same soil, with as heavy tamping as possible.

It contained 3,200 gm. Other pots were now filled with 2,900, 2,600, and 2,300 gm. of soil, four pots of each degree of compactness being used. Two pots of each series were steam-sterilized. Connecticut Havana tobacco was then transplanted into them. To avoid any abnormal conditions due to transplanting, especially in the compacted soils, which were so hard that a knife could scarcely be inserted into them, a small amount of soil was taken out of the center of each pot with a cork borer. The hole was filled with uninfested loose soil, and the young plants were transplanted into it so as to give all an equal chance to start.

The results were very interesting. The plants in the sterile compacted soils did very much better than was expected, although the loose soils were much more favorable to growth. In the infested soil, however, the plants in the compacted soil made no growth whatever (Pl. 5, IV). Examination of the roots showed that in the loose infested soil comparatively few lesions occurred, and the taproot was present, while with increasing compactness the taproot was lost, and gradually increasing numbers of lesions occurred.

The experiment has an important practical bearing on the preparation and cultivation of tobacco soils infested with *T. basicola*. Anything which can be done to avoid or remedy compactness or baking of soils will no doubt lessen the disease even in badly infested soils. In other words, soil in good tilth is less likely to be heavily damaged by *T. basicola* than soil in poor tilth.

TRANSPLANTING DISEASED SEEDLINGS

The influence of the use of diseased seedlings for transplanting to the field is not strictly an environmental feature of the problem. It relates, however, to the amount of infestation in the soil and has a very important practical bearing on results obtained under field conditions. It is, furthermore, a point upon which some contradictory evidence has been obtained by various experimenters, especially Benincasa (*1*), Gilbert (*12*), and Clinton (*8*). The results already presented, especially in regard to the influence of soil temperature, may serve to explain the variation in results from year to year, or of the recovery of infected transplanted seedlings. This is, however, apparently not the only explanation. The writer has shown that varieties of tobacco and even strains, vary in their resistance to rootrot (*14*). Transplanting healthy plants to infested soil under favorable environmental conditions for disease is shown to result in marked differences in yield of the different types used.

It is known that the tendency of diseased plants is to send out new roots to replace those lost by disease. Transplanting diseased plants consists practically in infesting a small area of soil surrounding the base of the plant with *T. basicola*. Part of the new roots, especially those

in the early stages of growth, must penetrate this infested soil before reaching large areas of uninfested soil. The ability to resist the disease will therefore determine roughly the number of roots and rapidity with which they pass through this infested area and become established, and should be roughly proportional to the resistance of the different varieties under similar environmental conditions.

To determine the influence of transplanting varieties differing widely in relative resistance to the rootrot, 11 such varieties were sown in a seed bed infested with *T. basicola*, and also in a sterilized bed as controls. The relative resistance in the seed bed is about the same as that in the field. The susceptible varieties especially did very poorly in the infested beds, but most of them reached a sufficient size for transplanting. About 40 plants of each variety from infested soil and the same number from uninfested soil, were then transplanted side by side in uninfested soil (Pl. 5, V). The results are given in Table XI in which the green weights of 25 healthy plants and 25 diseased plants of each variety are shown, together with the decreases in weight due to the use of infested seedlings. It will be noted that a reduced yield occurred in all cases, but whereas the disease was small in the case of varieties known to be resistant to *T. basicola* it was relatively very high in those varieties which are susceptible. The results are not exactly comparable on this basis on account of the difference in yield of varieties under normal conditions but serve to illustrate the point in question.

TABLE XI.—*Influence of transplanting diseased tobacco plants in uninfested soil*

Variety.	Weight of 25 green plants.		
	Healthy plants.	Infected plants.	Decrease due to disease.
	Gm.	*Gm.*	*Gm.*
White Burley	66. 50	25. 00	41. 50
Maryland Broadleaf	65. 00	38. 00	27. 00
Big Oronoco	57. 75	32. 50	25. 25
Yellow Pryor	59. 00	35. 50	23. 50
Pennsylvania Broadleaf	82. 50	27. 25	55. 25
Kentucky Greenleaf	49. 75	33. 50	16. 25
Italia Kentucky	60. 00	49. 00	11. 00
"Pease Seed"	49. 00	40. 00	9. 00
Ohio Seedleaf	70. 50	51. 00	19. 50
"Northern Hybrid"	65. 00	55. 00	10. 00
Brasile Beneventano	56. 50	53. 00	3. 50

Gilbert (*12*) reports an experiment in which "Havana Broadleaf" tobacco was used and in which the yield from infected and healthy plants was practically identical. By "Havana" Broadleaf was meant, it is presumed, the relatively resistant variety better known as "Connecticut Broadleaf." If environmental conditions were favorable for the

occurrence of disease, different results no doubt would have occurred had Gilbert used a more susceptible variety.

Practical advice on the use of infected seedlings will, then, vary with the variety used. Infected seedlings should never be used if it is possible to avoid it, especially on soils which are not infested, since this will only hasten the time when all the soil will become so thoroughly infested as to make a change to newer soils necessary. On the other hand, it frequently is necessary to risk infected plants, as others may be unobtainable. In such instances it is much less likely that serious injury will result if the infection is on a resistant variety. Again, it should be remembered that infected seedlings of even a susceptible variety transplanted into a heavily infested soil may produce a normal crop under favorable conditions, such as a very warm season and a relatively high soil temperature persisting for a long time.

SUMMARY

(1) The rootrot of tobacco, caused by *Thielavia basicola*, is marked by the stunting of plants in various degrees due to a reduced root system. The extent of the damage is determined in a large measure by the environmental conditions surrounding the roots of the host.

(2) A study of these environmental conditions is essential to the proper understanding of the occurrence and distribution of the disease in general and local areas, and to good judgment in recommendation for control measures.

(3) There seems to be no variation in the pathogenicity of the rootrot fungus secured from different sources. The amount of disease is determined entirely by the susceptibility of the host, the amount of infection, and the soil environmental factors surrounding the roots of the host.

(4) The factors especially studied were the amount of infestation in the soil, the soil moisture, soil temperature, soil reaction, physical structure, and fertility. An analysis of these factors separately as related to rootrot frequently is very difficult, if not impossible. Under normal conditions the end result in injury by rootrot is the sum total of the favorable and unfavorable action of these factors on the disease. Some of these factors are much more important than others.

(5) Other factors aside, the extent of infection and injury from tobacco rootrot is directly proportional to the amount of infestation of the soil.

(6) Rootrot is seemingly capable of developing in relatively dry soils. Increasing the moisture content of the soil up to three-fourths of its water-holding capacity does not materially increase rootrot. Saturated soils are, however, considerably more favorable for the disease than unsaturated ones.

(7) The temperature of the soil is undoubtedly the most important factor determining the extent of the rootrot of tobacco, other factors

being equal. The most favorable temperature for the disease ranges from 17° to 23° C. Below 15° the disease is less marked, and above 26° the severity is gradually reduced, until at about 29° or 30° it has little or no influence. At 32° practically no infection occurs even in the most heavily infested soils. Soil temperature records in the field for four seasons indicate that occurrence of the disease under practical conditions is determined primarily by soil temperature.

(8) The disease is checked by very high soil acidity. Heavy infection can occur, however, in soils showing a considerable acid reaction. The results depend a great deal upon the susceptibility of the variety used in the test, the amount of infection, the soil temperature, and on other factors. The results of tests of Wisconsin tobacco soils indicate that the use of acid fertilizers will not reduce infection by *T. basicola*. Although alkaline soils are more favorable to disease than very acid ones, the use of lime on infested soils may not necessarily reduce the yield due to increased infection from *T. basicola*.

(9) The amount of organic matter present or introduced into the soil does not play a very important part in the amount of infection. High organic matter content, however, no doubt favors increased infestation and aids the fungus to persist in the soil. Where heavy inoculation is made, infection apparently occurs more readily in pure sand than in the presence of organic matter, but under conditions unfavorable for the parasite the amount of infestation is more rapidly reduced in soils lacking in organic matter.

(10) Clay soils as such seemingly are no more favorable for infection than sand, and under certain conditions possibly less so. Clay may, however, favor the persistence of the parasite in the soil, and may actually favor infection because of increased danger of saturation with water and because of the occurrence of lower temperatures than in sandy soils.

(11) Increasing the fertility of infested soil by pure chemicals is likely to cause increased stunting of growth rather than increased growth, especially if too high a concentration of soil solution results. Fertilizers applied to heavily infested soils under practical conditions seem to be largely wasted except in seasons in which such high temperatures result that the disease is held in check.

(12) Field observations and limited laboratory experiments seem to show that infested soils when compacted are more favorable for the disease than loose, open soil.

(13) Transplanting infected seedlings to an uninfested field is a bad practice, although recovery from the disease may occur. Such recovery, environmental conditions aside, is proportional to the resistance of the type used.

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PLATE 1

I.—Influence of amount of infestation on injury from tobacco rootrot: A, All uninfested soil; B, three-fourths uninfested soil; C, one-half uninfested soil; D, one-fourth uninfested soil; E, all infested soil.

II, III.—Influence of moisture content of soil on the amount of injury done by the tobacco rootrot; II, infested soil; III, uninfested soil (control series)—

1A, one-fourth saturation infested soil;
2A, one-half saturation infested soil;
3A, three-fourths saturation infested soil;
4A, full saturation infested soil;
1B, one-fourth saturation uninfested soil;
2B, one-half saturation uninfested soil;
3B, three-fourths saturation uninfested soil;
4B, full saturation uninfested soil.

IV.—Influence of soil temperature on the growth of tobacco in infested soil (jars to left of temperature labels) and in uninfested soil (jars to right of temperature labels) at temperatures of approximately 13°, 17°, 23°, 26°, and 36° C.

I
A
B
C
D
E
II
1A
2A
3A
4A
III
1B
2B
3B
4B
IV
13°
17°C.
23°C.
26°C.
36°C.

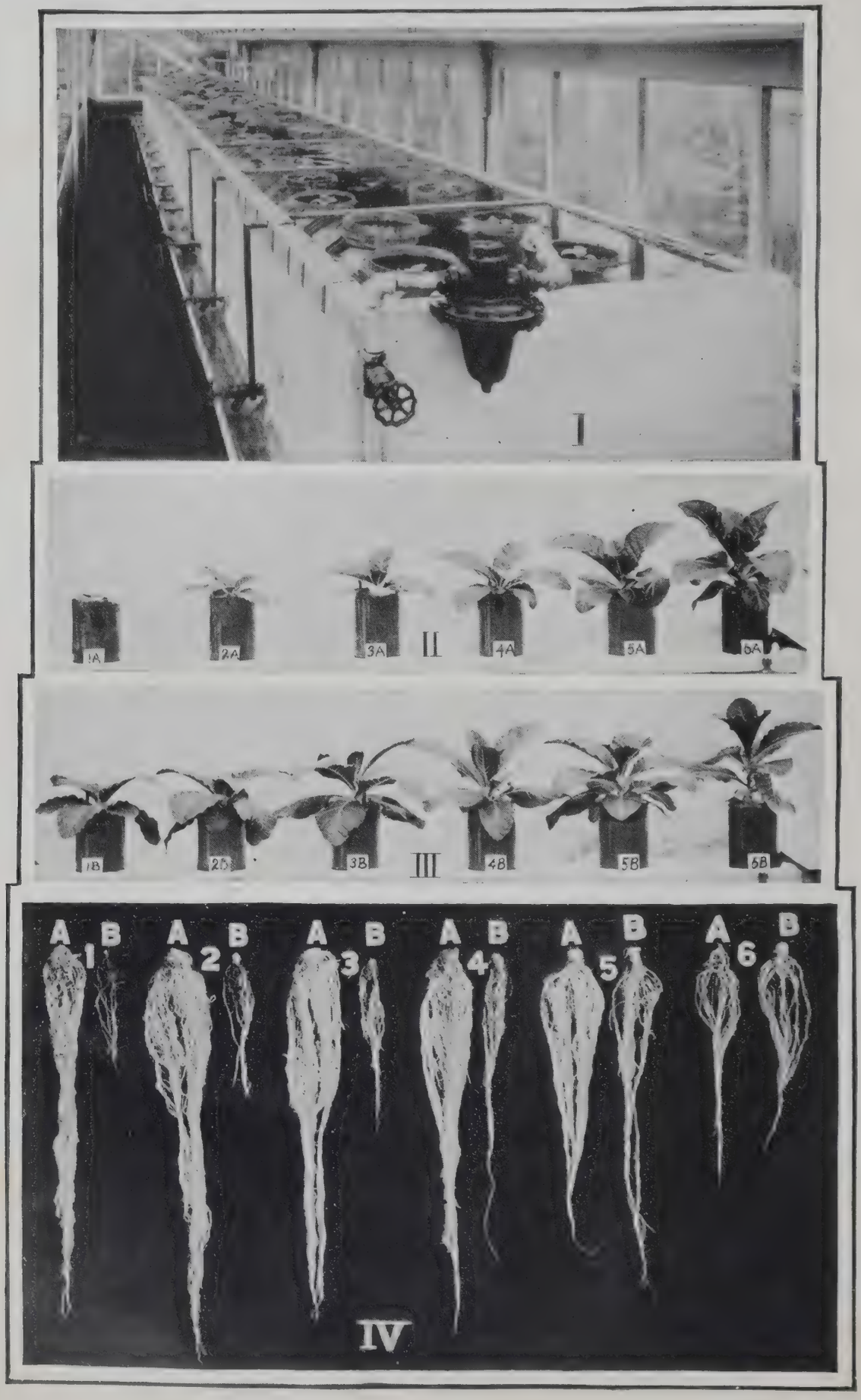

I
1A
2A
3A
II
4A
5A
6A
1B
2B
3B
III
4B
5B
6B
A B
1
A B
2
A B
3
A B
4
A B
5
A B
6
IV

PLATE 2

I.—Soil temperature tanks used in the temperature experiments. The water surface was later covered with sheet metal and asbestos board.

II, III.—Influence of soil temperature on the growth of tobacco:

1A, infested soil, 17°–18° C.;
2A, infested soil, 20°–21° C.;
3A, infested soil, 23°–24° C.;
4A, infested soil, 25°–26° C.;
5A, infested soil, 28°–29° C.;
6A, infested soil, 31°–32° C.;
1B, uninfested soil, 17°–18° C.;
2B, uninfested soil, 20°–21° C.;
3B, uninfested soil, 23°–24° C.;
4B, uninfested soil, 25°–26° C.;
5B, uninfested soil, 28°–29° C.;
6B, uninfested soil, 31°–32° C.

IV.—Influence of different soil temperatures on root development:

1A, uninfested soil, 17°–18° C.;
1B, infested soil, 17°–18° C.;
2A, uninfested soil, 20°–21° C.;
2B, infested soil, 20°–21° C.;
3A, uninfested soil, 23°–24° C.;
3B, infested soil, 23°–24° C.;
4A, uninfested soil, 25°–26° C.;
4B, infested soil, 25°–26° C.;
5A, uninfested soil, 28°–29° C.;
5B, infested soil, 28°–29° C.;
6A, uninfested soil, 31°–32° C.;
6B, infested soil, 31°–32° C.

PLATE 3

Influence of high (30° C.) and low (20° C.) soil-temperature on recovery of plants in infested soil. Both plants were taken from the field where they had made very little growth during the season and placed in temperature control tanks, the roots at 30° being like those at 20° at the beginning of the experiment.

30° C.
20° C.

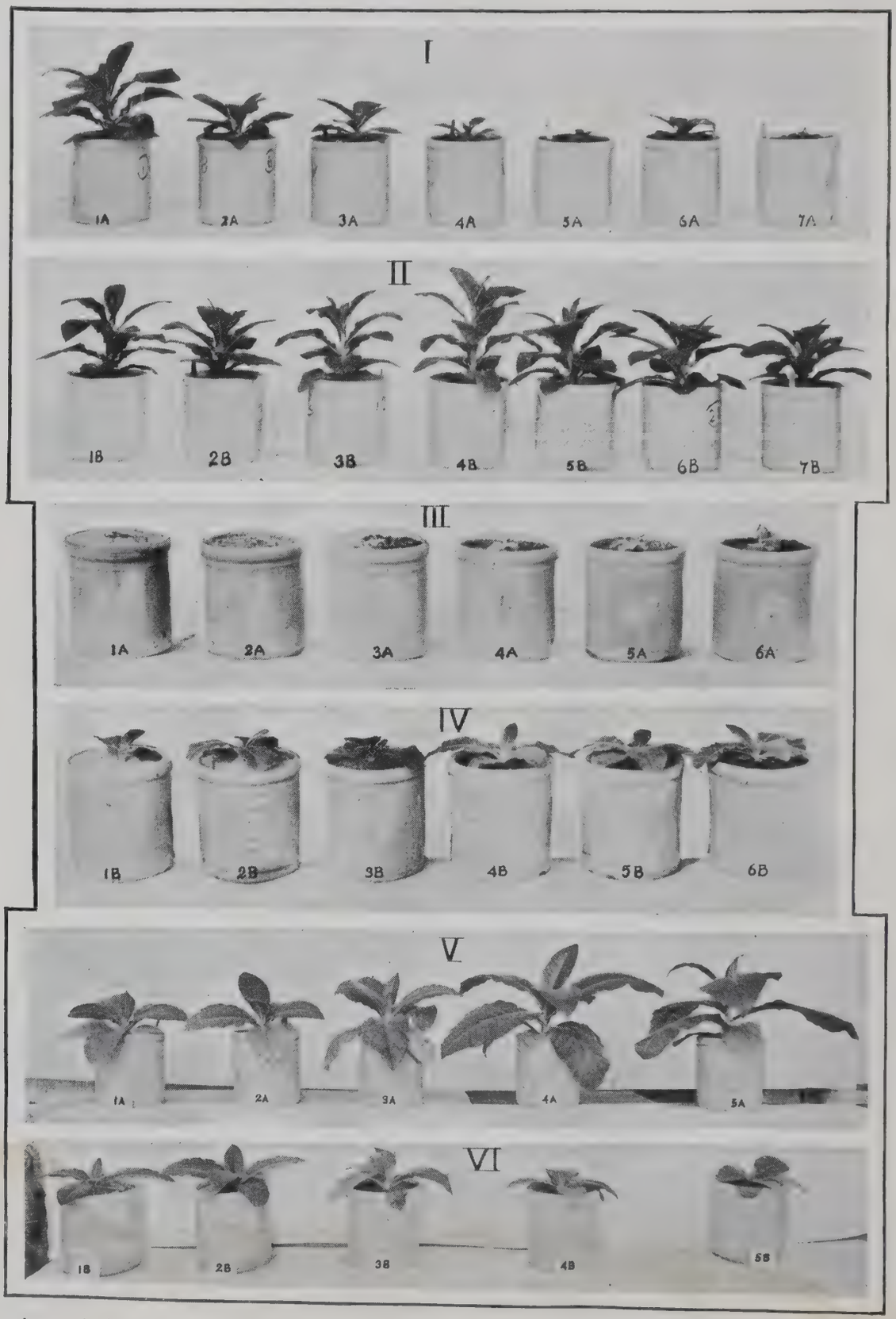

I
1A
2A
3A
4A
5A
6A
7A
II
1B
2B
3B
4B
5B
6B
7B
III
1A
2A
3A
4A
5A
6A
IV
1B
2B
3B
4B
5B
6B
V
1A
2A
3A
4A
5A
VI
1B
2B
3B
4B
5B

PLATE 4

I, II.—Influence of soil reaction on extent of damage by tobacco rootrot: I, Infested soil; II, uninfested soil—

1A, infested soil, lime requirement 9.38 tons per acre;
2A, infested soil, lime requirement 7.19 tons per acre;
3A, infested soil, lime requirement 4.60 tons per acre;
4A, infested soil, lime requirement 2.62 tons per acre;
5A, infested soil, lime requirement 0.72 ton per acre;
6A, infested soil, slightly alkaline;
7A, infested soil, strongly alkaline;
1B, uninfested soil, lime requirement 9.38 tons per acre;
2B, uninfested soil, lime requirement 7.19 tons per acre;
3B, uninfested soil, lime requirement 4.60 tons per acre;
4B, uninfested soil, lime requirement 2.62 tons per acre;
5B, uninfested soil, lime requirement 0.72 ton per acre;
6B, uninfested soil, slightly alkaline;
7B, uninfested soil, strongly alkaline.

III, IV.—Influence of the amount of organic matter in the soil on injury by tobacco rootrot: III, 1A–6A, Influence of gradually increasing amounts of organic matter in infested soil from 1A, no organic matter, to 6A, all leaf mold.

Planted soon after heavy inoculation.

IV.—1B–6B, Influence of gradually increasing amounts of organic matter in uninfested soil from 1B, no organic matter, to 6B, all leaf mold (control series).

Planted soon after heavy inoculation.

V, VI.—Influence of the amount of organic matter in the soil on injury by tobacco rootrot: V, 1A–5A, Influence of gradually increasing amounts of organic matter in uninfested soil from 1A, no organic matter, to 5A, all leaf mold (control series); VI, 1B–5B, Influence of gradually increasing amounts of organic matter in infested soil from 1B, no organic matter, to 5B, all leaf mold.

Planted some months after moderate inoculation.

PLATE 5

I.—Influence of relative amount of sand and clay on tobacco rootrot: A, uninfested series: B, infested series—

1 A, uninfested soil, three-fourths clay and one-fourth sand;
1 B, infested soil, three-fourths clay and one-fourth sand;
2 A, uninfested soil, one-half clay and one-half sand;
2B, infested soil, one-half clay and one-half sand;
3A, uninfested soil, three-fourths sand;
3B, infested soil, three-fourths sand;
4A, uninfested soil, all sand;
4B, infested soil, all sand.

II, III.—Influence of soil fertility on amount of tobacco rootrot: II, infested series; III, uninfested series—

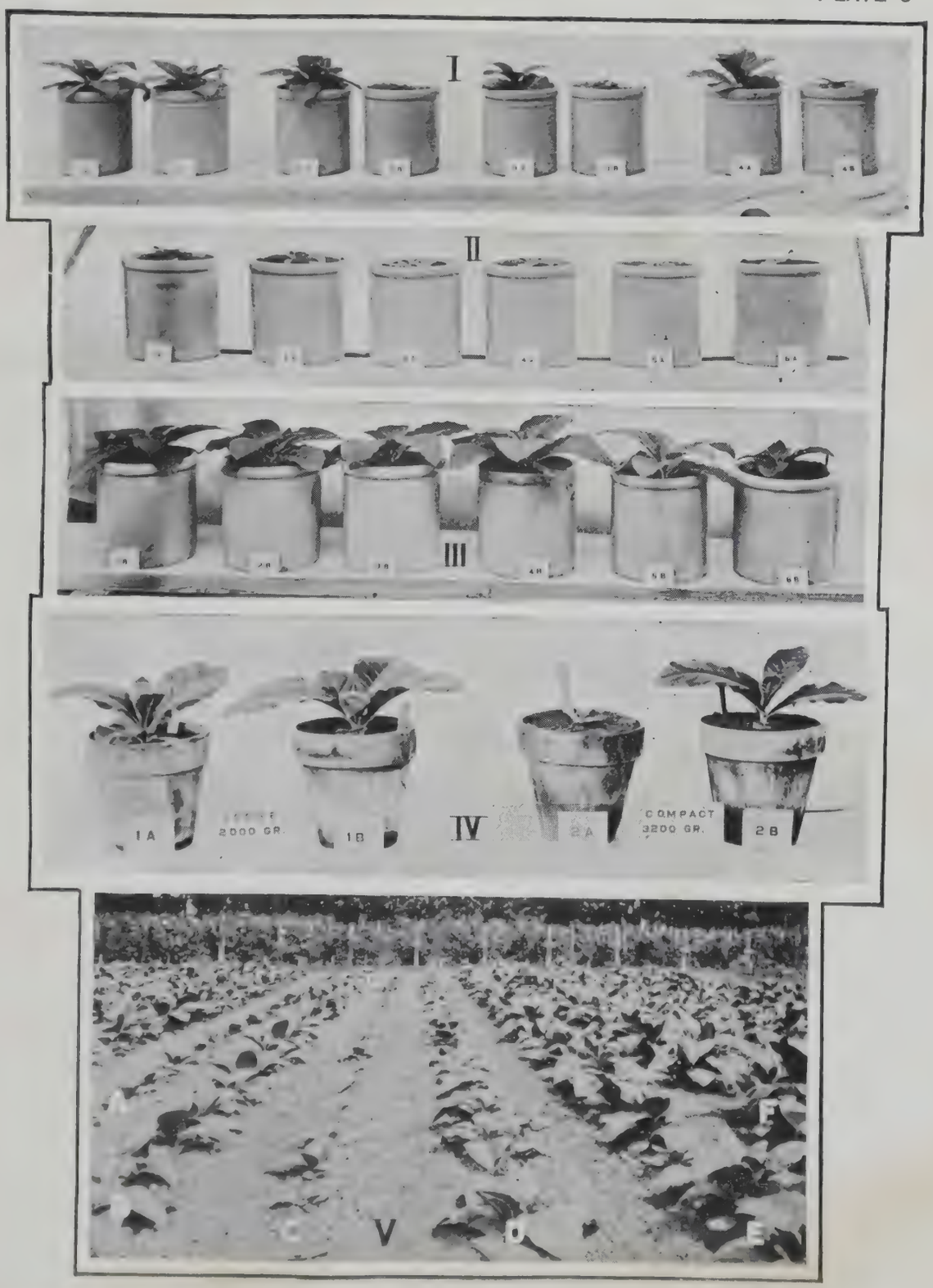
1A, infested soil, no treatment;
2A, infested soil, 3.5 gms. of nutrient salts;
3A, infested soil, 7.0 gms. of nutrient salts;
4A, infested soil, 14.00 gms. of nutrient salts;
5A, infested soil, 28 gms. of nutrient salts;
6A, infested soil, 56 gms. of nutrient salts.
Note increasing injury from nutrient salts beginning at pot 3A.
1B, uninfested soil, no treatment;
2B, uninfested soil, 3.5 gms. of nutrient salts;
3B, uninfested soil, 7.0 gms. of nutrient salts;
4B, uninfested soil, 14.00 gms. of nutrient salts;
5B, uninfested soil, 28 gms. of nutrient salts;
6B, uninfested soil, 56 gms. of nutrient salts.
Note injury from nutrient salts in pots 5B and 6B.

IV.—Relation of compactness of soil to injury caused by *Thielavia basicola:*

1A, infested soil, loosely packed;
1B, uninfested soil, loosely packed;
2A, infested soil, very compact;
2B, uninfested soil, very compact.

V.—Influence of transplanting infected seedlings in healthy soil:

A, Pennsylvania Broadleaf infected seedlings;
B, Pennsylvania Broadleaf healthy seedlings;
C, White Burley infected seedlings;
D, White Burley healthy seedlings;
E, Northern Hybrid (a resistant type) infected seedlings;
F, Northern Hybrid (a resistant type) healthy seedlings.


I
II
III
IV
1A
2000 GR.
1B
2A
COMPACT
3200 GR.
2B
V
C
D
E
F

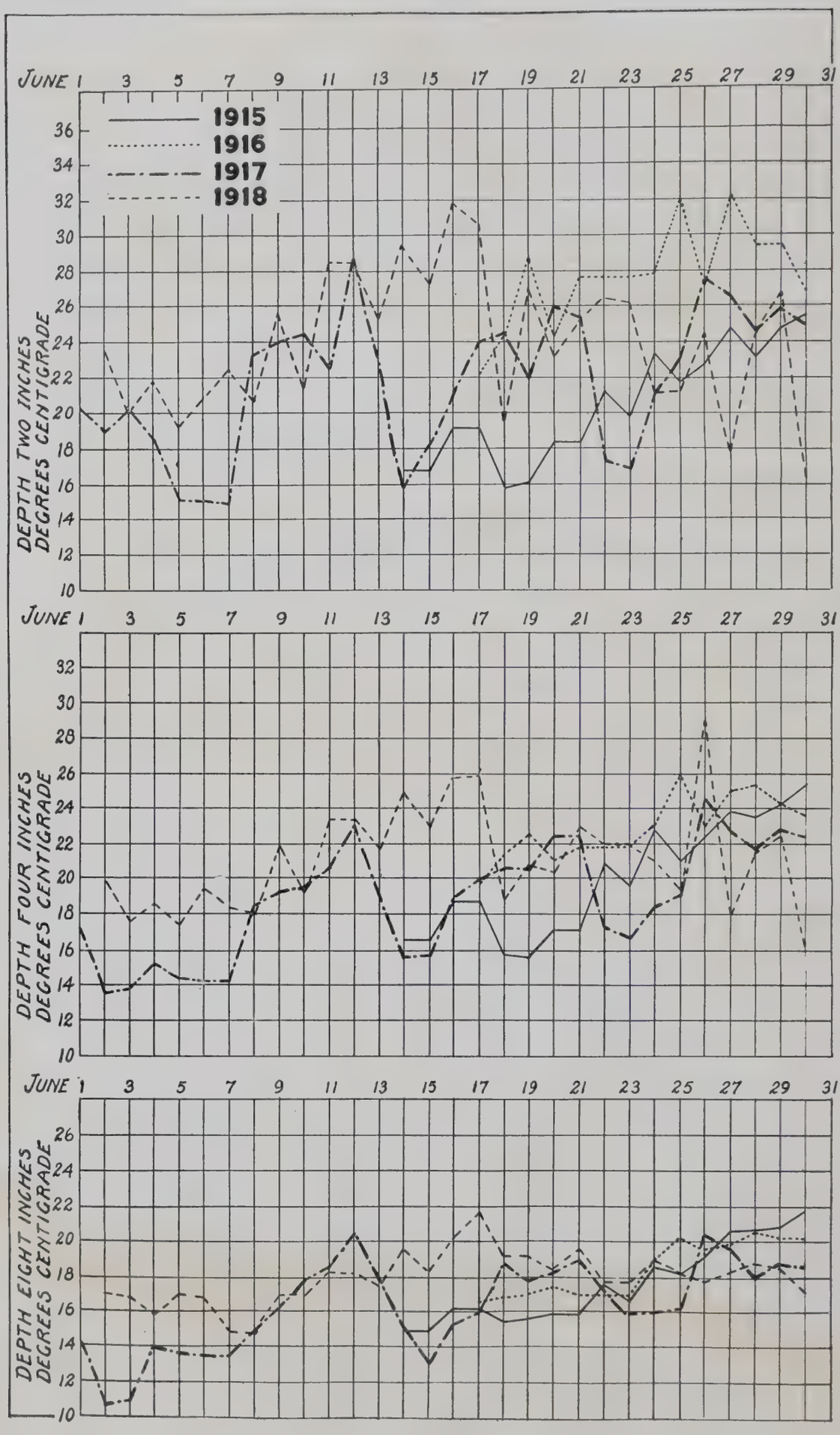
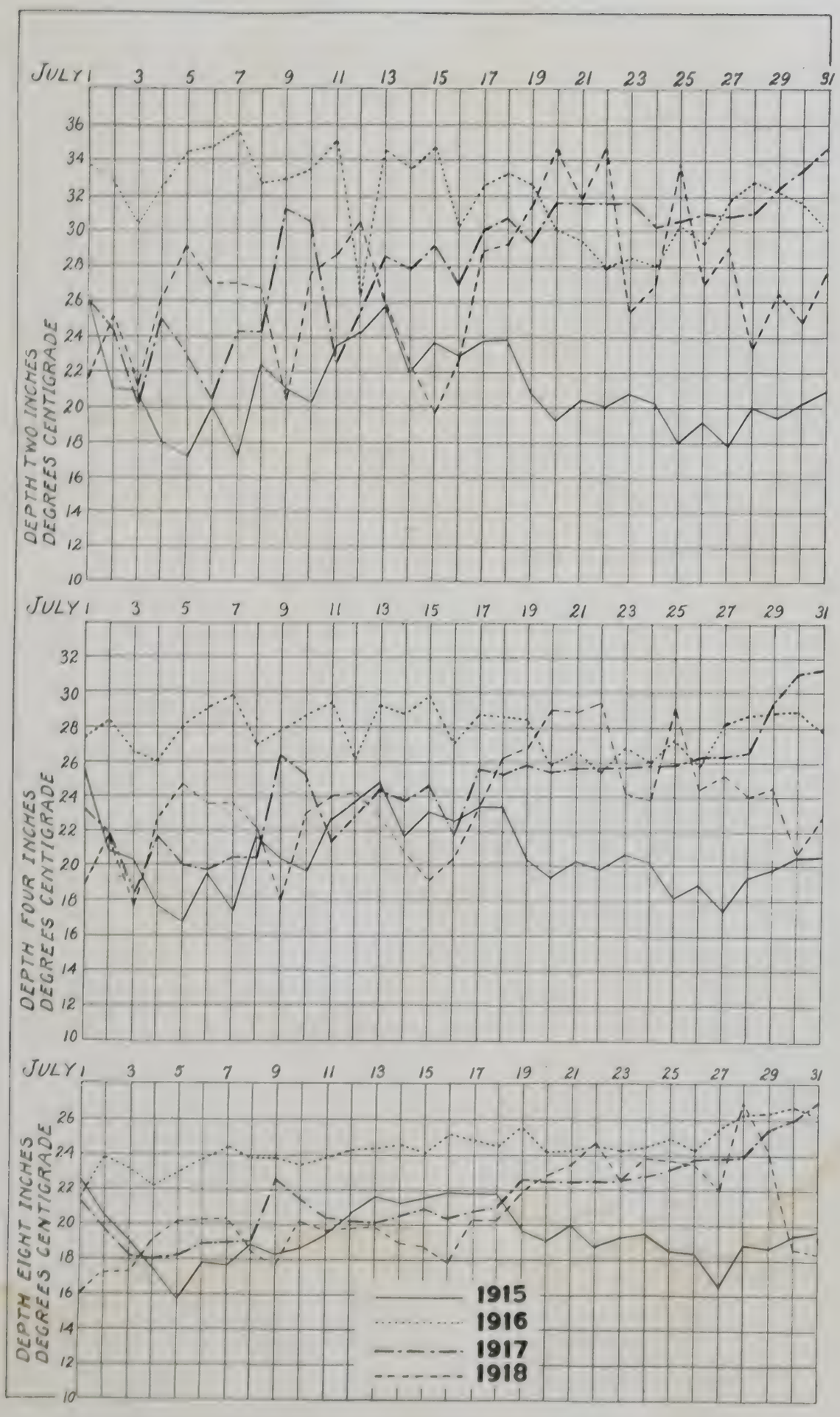

JUNE 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
1915
1916
1917
1918
DEPTH TWO INCHES
DEGREES CENTIGRADE
36 34 32 30 28 26 24 22 20 18 16 14 12 10
JUNE 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH FOUR INCHES
DEGREES CENTIGRADE
32 30 28 26 24 22 20 18 16 14 12 10
JUNE 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH EIGHT INCHES
DEGREES CENTIGRADE
26 24 22 20 18 16 14 12 10

PLATE 6

Soil temperature graphs for the month of June, 1915–1918, inclusive, at depths of 2, 4, and 8 inches.

PLATE 7

Soil temperature graphs for the month of July, 1915–1918, inclusive, at depths of 2, 4, and 8 inches.


JULY 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH TWO INCHES DEGREES CENTIGRADE
36 34 32 30 28 26 24 22 20 18 16 14 12 10
JULY 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH FOUR INCHES DEGREES CENTIGRADE
32 30 28 26 24 22 20 18 16 14 12 10
JULY 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH EIGHT INCHES DEGREES CENTIGRADE
26 24 22 20 18 16 14 12 10
1915
1916
1917
1918

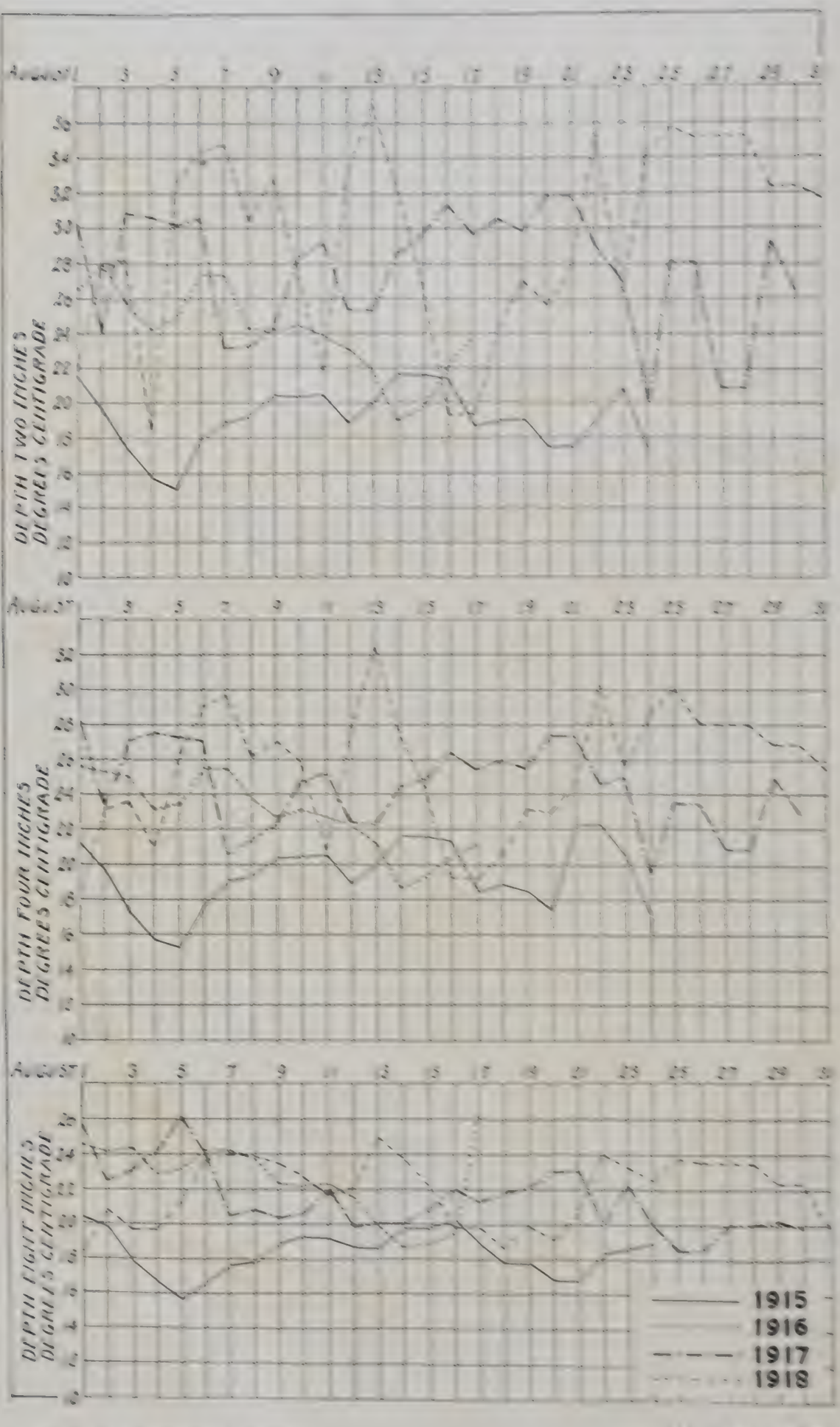

DEPTH TWO INCHES
DEGREES CENTIGRADE
DEPTH FOUR INCHES
DEGREES CENTIGRADE
DEPTH EIGHT INCHES
DEGREES CENTIGRADE
1915
1916
1917
1918

PLATE 8

Soil temperature graphs for the month of August, 1915–1918, inclusive, at depths of 2, 4, and 8 inches.

ADDITIONAL COPIES

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RELATION OF SULPHATES TO PLANT GROWTH AND COMPOSITION

By H. G. MILLER,

Assistant Chemist, Oregon Agricultural Experiment Station

HISTORICAL REVIEW

The oxidation of sulphur in the soil and the relation of the products formed is plant growth, bacterial development and activity, and to the release of other forms of plant food have been reported upon by many investigators. In reviewing the work, many of the writers have reported beneficial results from the use of sulphur fertilizers, especially with those plants high in protein and other sulphur-containing compounds. Various views are given as to how sulphur functions in producing these increased yields. Analysis of soils reported by Hart and Peterson (*11*),[1] Shedd (*22, 23*), Brown and Kellogg (*7*), and Swanson and Miller (*26*), show a lower sulphur content in the cultivated soil as compared to the phosphorus, while many of the cultivated plants show a larger content of sulphur than phosphorus. These results indicate that sulphur would become a limiting factor before phosphorus.

It is generally concluded that sulphur to be available for plant food must be in the sulphate form, so that a soil having a high sulphur content may not necessarily supply enough sulphate sulphur for maximum growth. Brown and Kellogg (*6*) have shown that different soils have unlike sulphofying powers and some of the factors influencing the change of elemental sulphur and sulphides to sulphate form. In lysimeter experiments at Cornell, Lyon and Bizzell (*17*) report that the sulphate sulphur in the drainage water was from three to six times as great as in the crops and the sulphur content of the drainage water from the unplanted soil was about equal to the sulphur content of the crop and drainage water from the planted soil. Swanson and Miller (*26*) conclude from an investigation on sulphur in Kansas soils that—

> the loss in sulphur due to the amount taken up by the crop is insignificant as compared with the total amount which has disappeared from the soil. This means that sulphofication has been in excess of the needs of the crop, and the sulphates produced have been leached out of the ground.

[1] Reference is made by number (italic) to "Literature cited," pp. 100–102.

They report no increased yield when sulphur was applied as potassium sulphate (K_2SO_4), but state that the loss of sulphur can not continue without affecting crop yields. Hart and Peterson (*11*) calculate from data obtained at the Rothamsted Experiment Station, Harpenden, England, and the Wisconsin Experiment Station that the loss of sulphur in drainage water is three times that brought to an acre surface from the atmosphere.

Results of the investigations mentioned above show that the soil is capable of producing sulphate sulphur and that there is a tremendous loss of such sulphur in the drainage water. In certain cases no beneficial results have been obtained from sulphur fertilizers, which, is not surprising, but in many instances sulphur application has caused increased yields. No doubt, in many soils, if the supply of sulphate sulphur formed was retained for plant food, they would not respond to sulphate treatment but the continual loss of sulphate sulphur and the large amount needed by some plants require that additional sulphate sulphur be present during the growing period to obtain better growth. A soil with a high sulphur content may not supply enough sulphate sulphur in comparison to the other plant food to obtain the maximum growth, while conditions in a soil of lower sulphur content may be such as to supply an adequate amount of sulphate sulphur.

In addition to sulphur acting as a plant food, several other explanations have been given as to its action in the soil. Certain investigators, Bernhard (*3*), Chancrin and Desriot (*8*), say that it functions as a partial sterilizer, others that the sulphuric acid produced acts upon the mineral matter of the soil, rendering it more soluble. Lipman (*15*, *16*) and his coworkers have shown (*1*) that the oxidation of sulphur in sand and soils has acted upon the raw-rock-phosphate so as to increase the water and ammonium-citrate-soluble phosphorus (*2*); that the formation of sulphate sulphur paralleled the increase of available phosphoric acid (*3*); and that the sulphur-floats-soil compost could be employed as a substitute for acid phosphate for plant growth. Brown and Gwinn (*5*) have found that the addition of sulphur to the soil increased the availability of raw-rockphosphate, the gain being greatest where manure and sulphur were used together. McLean (*18*) in a number of experiments has shown the conditions which are more favorable for the oxidation of sulphur by microorganisms and production of available phosphorus. Lipman (*14*) suggests that the sulphuric acid may act in making alkali areas productive by converting sodium carbonate into sodium sulphate. The favorable results obtained by adding gypsum have often been attributed to the calcium liberating potassium, but the experiments of Hart and Tottingham (*12*) show that a complete fertilizer plus calcium sulphate gave increased yields over those obtained with a complete fertilizer containing potassium chlorid, and that here the action of calcium sulphate must have been direct.

The action of sulphates upon soil bacteria has also been studied. Fred and Hart (*9*) have shown small increases in the number of soil bacteria and a slight increase in ammonification and carbon-dioxid evolution by adding certain sulphates to the soil. A bacteriological investigation by Janicaud (*13*) indicated that sulphur had a favorable influence on the development of bacteria in the soil. Pitz (*20*) reports no marked effect on the number of bacteria found on agar plates, but he does report an increase in legume bacteria from the use of calcium sulphate. Elemental sulphur caused a decrease in the total number of bacteria that grow on agar plates, but an increase in ammonification was accompanied by a parallel decrease in nitrate formation. Boullanger and Dugardin (*4*) state that the presence of small amounts of sulphur materially increases the activity of the ammonifying bacteria. Ames and Richmond (*2*) conclude from experimenting on relation of sulphofication to nitrogen transformation that the increase in ammonia which accompanied the decrease in yield of nitrates when sulphur was oxidized can not be considered as indicative of sulphofication having exerted a stimulating effect on ammonification. A deficiency of base in the soil allows the ammonia formed to neutralize the sulphur and it remains as ammonium sulphate.

Certain soils in Oregon have responded greatly to the use of sulphur and its compounds. In this locality greater crop production in many cases has resulted from using gypsum than in using lime. Reimer (*21*) of the Southern Oregon Experiment Station has obtained large increases in alfalfa yield by the use of elemental sulphur. This marked effect from the use of sulphur fertilizers suggested that it would be of interest and practical value to carry on some greenhouse experiments in order to study the effect of sulphur on early growth and composition of the plants, and also to attempt to determine whether it acted directly in supplying the plant with food or functioned in some other way.

PLAN AND OBJECT OF THE EXPERIMENT

For this work three Oregon soils, classified as a beaverdam, a Medford loam, and an antelope-clay-adobe, and designated as A, B, and C, respectively, in the tables, were chosen. The first was taken mainly for its high sulphur content, the second one because it did not respond to sulphur treatment in the field, while the third did respond to elemental sulphur fertilizer. The results obtained on analysis of these soils are given in Table I. The plants chosen were red clover, oats, and rape.

TABLE I.—*Percentage of constituents foun in soils*

Constituent.	Soil A.[b]	Soil B.[b]	Soil C.[b]
Potassium oxid (K_2O)	0.32	1.25	0.60
Phosphorus pentoxid (P_2O_5)	.349	.160	.135
Sulphur	.183	.034	.027
Calcium oxid (CaO)[a]	.....	4.02	3.24
Calcium carbonate ($CaCO_3$)[a]	.....	None.	.11

[a] Taken from analyses of soil sample when the field experiments were carried on.
[b] Soil A=beaverdam; soil B=Medford loam; soil C=antelope-clay-adobe.

It was decided to apply sulphur in the form of sodium sulphate (Na_2SO_4), calcium sulphate ($CaSO_4$), and elemental sulphur. The elemental sulphur was mixed with the soil at the time of planting, but the sulphates were added daily in the form of a solution. This daily addition of sulphates maintained a continuous supply of sulphates for the plant and it was thought that through the growth of the plants in the pots receiving the different forms of sulphur one could ascertain whether the elemental sulphur was able to supply the necessary sulphate. In order to eliminate nitrogen as a limiting factor, sodium nitrate ($NaNO_3$) in solution was added daily. By keeping up an available supply of nitrogen, a study could be made of the influence of sulphur fertilizer on the amount of nitrogen taken up by the plant. Nitrogen and sulphur enter into the composition of proteins and mustard oils so that an abundant supply of sulphates and nitrates in the plant may increase the elaboration of those organic compounds containing sulphur and nitrogen.

In addition to growing the plants on soil, they were also grown in sand pots receiving extract from the soil plus any nutrient that was added to the soil. For example, for a soil receiving a solution containing calcium sulphate and sodium nitrate, there was a corresponding sand pot receiving a water extract of the soil plus calcium sulphate and sodium nitrate. The following statement shows what each pot growing clover received:

SOIL

Pot 1:
- Calcium sulphate.
- Sodium nitrate.

Pot 2:
- Sodium sulphate.
- Sodium nitrate.

Pot 3:
- Sodium nitrate.
- Calcium carbonate.
- Sulphur.

Pot 4: Sodium nitrate.

Pot 5:
- Sodium nitrate.
- Calcium carbonate.

Pot 6: No fertilizer.

SAND

Pot 7:
- Soil solution.
- Calcium sulphate.
- Sodium nitrate.

Pot 8:
- Soil solution.
- Sodium sulphate.
- Sodium nitrate.

Pot 9:
- Soil solution.
- Sodium nitrate.
- Calcium carbonate.
- Sulphur.

Pot 10:
- Soil solution.
- Sodium nitrate.

Pot 4 (Pl. 9, A, B, and C) is a control to compare with 1 and 2, and 5 is a control on pot 3. Pot 10 receives no sulphur except that in the original soil extract. The pots growing oats and rape were treated in the same way, and this was repeated for each soil, making in all 90 pots (Pl. 9–12).

Hall, Brenchley, and Underwood (*10*) at the Rothamsted Experiment Station, in some experimental work in support of the theory of the direct nutrition of plants by fertilizers, found that wheat and barley showed parallel growth in the soil, in the soil extracts, and in artificial solutions of the same phosphorus-pentoxid (P_2O_5) and potassium-oxid (K_2O) content. The soil solutions corresponded to the natural drainage water, depending upon the past fertilizing treatment and present composition of the soil. The growth in extracts from poorly fertilized soils could be made equal by direct addition of suitable phosphate and potassium salts. Boiling did not affect the nutritive value of the solutions, and the diffusion of the nutrient solution over particles of sand did not interfere with the growth, although proper aeration of the roots was found to be essential.

By growing the plants on sand the possibility of the sulphates acting on minerals in the soil would be eliminated, and boiling the soil extract would destroy the bacteria originally in the solution. So in these experiments, if an increase in growth is observed in the soil pots from the application of sulphur fertilizer, and a corresponding increase is also obtained in the sand pots containing soil extract to which sulphur had been added, this same order of growth in the soil and sand would indicate in all probability that sulphur had acted directly in promoting the growth.

The object of this experiment was to make a study of the influence of an available supply of sulphates on the early growth of the plants and determine whether the elemental sulphur was capable also of supplying the necessary sulphates; to see what effect sulphates would have on the nitrogen content of the plant and if beneficial results are obtained whether the sulphur acted directly as a plant food in producing them.

EXPERIMENTAL WORK

The pots used were ordinary clay flowerpots which had been paraffined on the inside, and each contained about 700 gm. of soil. The sand was of a fine quality, obtained from Eimer and Amend. It was washed with dilute hydorchloric acid until no potassium, phorphorus, or sulphur was detected in the acid extract. Larger pots were not used on account of the beginning of this type of experiment, the number of pots needed and the individual attention required. The growing period was about two months with the exception of the oats which were allowed to ripen. The seed was sown on March 15, and the work was carried on in the greenhouse. At the end of a month the plants were thinned out so that there were 10 clover plants, 6 oat plants, and 3 rape plants per pot. At this time the sulphur and nitrogen were determined in the clover plants taken from soil A. The clover and rape were cut on June 1, the dry weight taken, and the total sulphur and nitrogen determined. Twenty-five cc. of nutrient solutions containing compounds as given in the statement on p. 90 were added daily to each pot after growth had started.

Where elemental sulphur and calcium carbonate ($CaCO_3$) were added, 0.3 gm. and 1 gm., respectively, of the above substances were mixed with the soil or sand in each pot before planting. In the control receiving no added nutrients, 25 cc. of distilled water was added. The concentration of the salts per liter of solution were as follows:

	Gm. per liter.
Sodium nitrate	0.25
Sodium sulphate	.20
Calcium sulphate	.25

The salts were dissolved in the same solution when more than one salt was added to a pot. The sand cultures received the soil extract which contained the additional nutrients as required. When the cultures required further moisture, the same amount of water was added to each of the pots. The soil extract was prepared by thoroughly mixing one part of soil with two of water. The solution was allowed to stand over night and then filtered through a porcelain filter. The clear filtrate was sterilized by boiling for 15 minutes. Analysis of the soil solution as given in Table II was made according to a method given by Stewart (*25*).

TABLE II.—*Soil constituents found in soil extracts expressed as parts per million of the soil extract*

Soil.	Potassium oxid.	Phosphorus pentoxid.	Sulphate sulphur.	Total sulphur.	Calcium oxid.
A[a]	10.0	3.2	7.4	9.6	23.0
B[a]	19.8	3.4	3.6	3.6	15.0
C[a]	7.2	1.0	3.2	3.2	22.0

[a] Soil A=beaverdam; soil B=Medford loam; soil C=antelope-clay-adobe.

DISCUSSION OF RESULTS

On examining the data in Table III it is noted that the weight of the straw grown on soils B and C and receiving sulphur fertilizer is greater than where no sulphur was used. An increase in weight of the crops is also observed in the sand pots receiving additional sulphur over those receiving soil extract plus sodium nitrate. The absence of plant food in the original sand and the use of sterilized soil extract shows undoubtedly that sulphur acted directly in promoting this growth. The same response of the soil to sulphur leads one to conclude that the sulphur here too has acted directly in promoting the growth. This increase in growth is also accompanied by an increase in sulphur content of the oat straw. In soil A this increase from sulphur application is not obtained. The weight of straw from the pots receiving calcium sulphate, sodium sulphate, and sulphur in addition to sodium nitrate is no greater than from the one receiving sodium nitrate only. In the sand pots receiving added sulphur we find no increase in weight of straw over the one receiving no extra sulphur. This is not surprising when the sulphur content

of the soil extracts is compared. The soil solution from A contains twice as much sulphate sulphur as the soil extracts from B and C and the total sulphur is three times as great. Apparently there is enough sulphur in the soil solution compared to the other elements for straw production. The development of the oat seed as shown in Table IV, agrees very well with the weight increases of the straw as shown in Table III. In comparing the growth of the oats on the soils receiving sodium nitrate plus calcium carbonate to those receiving sodium nitrate only, the calcium carbonate appears to have an injurious effect upon growth, but in comparing the calcium carbonate-sodium-nitrate-treated soils to those receiving sulphur, sodium nitrate, and calcium carbonate, the sulphur has caused increased growth in all cases.

TABLE III.—*Weight of oat straw and its percentage of sulphur and nitrogen on the different soils and sand cultures obtained from the different fertilizer treatment*

Treatment.	Soil.[a]								
	A.			B.			C.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*			*Gm.*		
Calcium sulphate..... Sodium nitrate.....	2.21	0.346	0.28	1.92	0.45	0.21	2.01	0.175	0.32
Sodium sulphate..... Sodium nitrate.....	2.13	.282	.33	1.90	.42	.266	2.01	.113	.33
Sulphur..... Sodium nitrate..... Calcium carbonate....	2.22	.370	.42	1.96	.58	.336	1.75	.30	.21
Sodium nitrate.....	2.23	.016	.17	1.41	.....	.476	1.68	.005	.54
Sodium nitrate..... Calcium carbonate....	1.83	.....	.29	1.34	.....	.672	.63	.....	.....
No fertilizer.....	.97	.143	.66	1.67	.....	.806	.54	.....	.49

Treatment.	Sand.[a]								
	Extract A.			Extract B.			Extract C.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*			*Gm.*		
Calcium sulphate..... Sodium nitrate.....	1.69	0.16	1.30	1.72	0.21	0.92	1.62	0.33	0.80
Sodium sulphate..... Sodium nitrate.....	1.63	.30	1.10	1.57	.13	.98	1.53	.34	.96
Sulphur..... Sodium nitrate..... Calcium carbonate....	1.48	.42	1.54	1.36	.32	1.29	1.76	.39	.73
Sodium nitrate.....	1.59	.13	1.50	1.06	.015	1.46	1.08	.05	1.70

[a] A=beaverdam soil or sand; B=Medford loam; C=antelope-clay-adobe.

TABLE IV.—*Weight of oat seed grown on soil and sand cultures*

[Percentage of nitrogen is given on oats grown in soils A and B]

Treatment.	Soil.[a]					Sand.[a]		
	A.		B.		C.	Weight.		
	Weight.	Percentage of nitrogen.	Weight.	Percentage of nitrogen.	Weight.	Extract A.	Extract B.	Extract C.
	Gm.		*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*
Calcium sulphate Sodium nitrate	1.63	1.72	1.25	1.73	0.85	0.67	0.71	0.64
Sodium sulphate Sodium nitrate	1.44	1.79	1.13	1.71	.92	.77	.55	.48
Sulphur Sodium nitrate Calcium carbonate	1.45	1.46	.96	1.79	.82	.67	.38	.72
Sodium nitrate	1.62	1.73	.76	2.33	.61	.55	.36	.26
Sodium nitrate Calcium carbonate	1.06	1.77	.52	2.53	.08	.....	.....	.....
No fertilizer	.34	2.29	.27	2.33	.10	.....	.....	.....

[a] A=beaverdam soil or sand; B=Medford loam; C=antelope-clay-adobe.

The results on clover as given in Table V show increased yields in all cases where sulphur was added to the soils. This increase is also seen on the sand cultures receiving soil solutions from soils B and C, but not on the sand receiving nutrients as soil A. The oats grown on soil A, as on the sand receiving extract from A, did not respond to sulphur application, but the clover did respond to sulphur treatment of the soil. However, the clover growing on the sand and receiving its plant food from the soil extract did not show increased growth where sulphur was added. Naturally one may attribute this difference to some other factor present in the soil which was favorably influenced by the sulphur. Omitting this important factor and observing the weights of the dry material grown in the soil solution, there appears to be enough available sulphur in soil A and in the soil extract to eliminate sulphur as being directly a limiting factor as a plant food, while in the other soils the supply of available sulphur seems to be limited in comparison to the other plant food available.

It is probable that the other factors acting here are the legume bacteria which are present in the soil but not in the sand. The data in Table VI show the increase in weight of the roots where sulphur was used, and when the roots were examined the number of nodules, according to estimate, varied directly as the weight of the roots. The roots of those plants grown in the sand, of course, contained no nodules. Another reason why the bacteria appear to be favorably influenced by the sulphur is the noticeable percentage of increase of nitrogen in those plants grown on soil receiving sulphur while the plants grown on sand do not show this increase in nitrogen content. For this short period of growth the sulphur apparently has a marked influence on the nitrogen content of the clover.

TABLE V.—*Weight of clover and its percentage of sulphur and nitrogen on the different soils and sand cultures obtained from the different fertilizer treatments*

Treatment.	Soil.[a]								
	A.			B.			C.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*			*Gm.*		
Calcium sulphate, Sodium nitrate	3.98	0.197	2.94	2.20	0.227	2.78	1.69	0.240	2.67
Sodium sulphate, Sodium nitrate	4.06	.181	2.93	1.37	.280	2.95	1.66	.205	3.33
Sulphur, Calcium carbonate, Sodium nitrate	3.89	.198	3.10	1.87	.234	3.64	.65	.....	1.28
Sodium nitrate	1.76	.097	2.32	.99	.056	2.19	.62	.....	2.10
Calcium carbonate, Sodium nitrate	1.48	.012	1.98	.61	.032	1.92	.42	.....	1.79
No fertilizer	1.80	.055	1.91	.65	.037	2.58	.49	.....	1.62

Treatment.	Sand.[a]						
	Extract A.		Extract B.		Extract C.		
	Weight.	Percentage of nitrogen.	Weight.	Percentage of nitrogen.	Weight.[b]	Percentage of sulphur.	Percentage of nitrogen.
	Gm.		*Gm.*		*Gm.*		
Calcium sulphate, Sodium nitrate	0.54	2.38	0.38	1.8	1.0	0.467	2.11
Sodium sulphate, Sodium nitrate	.58	2.56	.36	2.14	.....	.....	.....
Sulphur, Calcium carbonate, Sodium nitrate	.49	2.54	.24	1.92	1.20	.493	2.42
Sodium nitrate	.63	2.35	.22	1.90	.52	.....	1.86
Calcium carbonate, Sodium nitrate	.....	.....	.....	.....	.....	.....	.....
No fertilizer	.....	.....	.....	.....	.....	.....	.....

[a] A=beaverdam soil or sand; B=Medford loam; C=antelope-clay-adobe.
[b] Plants were not thinned out. There was no growth in sodium-sulphate sodium-nitrate pot.

It appears that the sulphur increases the nitrogen content by stimulating the activity of the legume bacteria causing greater nitrogen fixation. The total nitrogen removed from the sulphured soils is three times as great as from the unsulphured soils. These plants, of course, have grown for only two months and whether the mature plant would show this same ratio will have to be decided by further experiments. In comparing the nitrogen and sulphur contents of the clover grown in soil A at two different periods as given in Table VII, there is a decrease in percentage of nitrogen and sulphur from May 1 to June 1. Perhaps the percentage

of total sulphur and nitrogen would grow less as the plant developed, until, at maturity, the nitrogen content would average about the same for all the clover grown under the different fertilizer treatments. In certain pots, maturity, undoubtedly, would be reached sooner, but allowing each group to grow until they all reached the same stage of development, it would be of importance to know whether the sulphur had affected the nitrogen content and the character of the compounds containing nitrogen. Samples of alfalfa grown on sulphur-fertilized soils in Oregon,[1] as shown in Table IX, have shown a higher nitrogen content than those grown on the same soil without sulphur application. Shedd (*24*) reports increase in protein content of soybeans from ammonium-sulphate fertilizer and Ames and Boltz (*1*) report larger protein content in rape where sulphates were in the fertilizer used. In this experiment available nitrogen was present in the form of nitrates. It would be of interest to know whether by maintaining the sulphate supply an increase in nitrogen assimilation from the air could be brought about.

TABLE VI.—*Weight of clover roots expressed in gms. grown on the different soils receiving various treatments*

Treatment.	Soil A.[a]	Soil B.[a]	Soil C.[a]
Calcium sulphate, Sodium nitrate	1.15	0.85	0.44
Sodium sulphate, Sodium nitrate	.95	.36	.28
Sulphur, Calcium carbonate, Sodium nitrate	.93	.49	.10
Sodium nitrate	.60	.33	.18
Sodium nitrate, Calcium carbonate	.53	.23	.10
No fertilizer	.51	.21	.13

[a] Soil A=beaverdam; soil B=Medford loam; soil C=antelope-clay-adobe.

TABLE VII.—*Sulphur and nitrogen content at different stages of growth in clover grown on soil A*

Treatment.	Percentage of sulphur, May 1.	Percentage of sulphur, June 1.	Percentage of nitrogen, May 1.	Percentage of nitrogen, June 1.
Calcium sulphate, Sodium nitrate	0.285	0.197	3.14	2.94
Sodium sulphate, Sodium nitrate	.260	.181	3.57	2.93
Sulphur, Sodium nitrate, Calcium carbonate	.360	.198	3.24	3.10
Sodium nitrate	.086	.097	2.80	2.32
Sodium nitrate, Calcium carbonate	.070	.012	2.13	1.98
No fertilizer	.130	.055	2.67	1.91

[1] Reimer and Tartar. Unpublished data, Oregon Agricultural Experiment Station.

The rape plant did not show this general response to sulphur treatment like the other plants, for in several instances the growth is greater in those pots receiving no sulphur. However, if a comparison is made between the soil and sand pots receiving sodium nitrate and those receiving sodium nitrate plus sodium sulphate, it is observed from the data in Table VIII that increased growth has resulted from the addition of sodium sulphate on both the sand and the soil. The growths of rape on the soils and their extracts parallel each other very well. The rape grew very poorly on the extract from soil C, so no data are given. Where a comparison is made on the growths of the crops on the different soils they do not follow the same order, and the sulphur and nitrogen content do not show the same change from the different fertilizer treatments; but the rape grown in pots receiving sulphur, sodium nitrate, and calcium carbonate has a higher percentage of sulphur than that grown in the other pots, yet the total sulphur removed is not much larger. The plants grown on the sand have higher percentages of nitrogen and sulphur but the total sulphur and nitrogen removed is no greater than for those plants grown in the soil.

The total sulphur present in plants is far greater where sulphur fertilizer was used. On account of the small amount of material, the sulphate sulphur was not determined so that it is not possible to tell whether the organic sulphur was increased. Analysis, in this laboratory, for organic sulphur and sulphate sulphur in alfalfa hay grown on soils receiving 300 pounds of sulphur per acre and on the same soils receiving no sulphur fertilizer as given in Table IX shows that the organic sulphur was increased by the application of sulphur. Shedd (*24*) found an increase in organic sulphur in soybeans from the use of ammonium sulphate fertilizer.

TABLE VIII.—*Weight of rape and its percentage of sulphur and nitrogen on the different soils and sand cultures*

Treatment.	Soil.[a]								
	A.			B.			C.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*			*Gm.*		
Calcium sulphate..... Sodium nitrate.....	1.81	0.75	1.39	1.57	0.66	0.903	1.98	0.61	1.06
Sodium sulphate..... Sodium nitrate.....	2.92	.50	1.65	2.26	.55	.990	2.31	.65	.99
Sulphur..... Sodium nitrate..... Calcium carbonate....	1.03	1.19	1.29	1.89	.80	1.47	1.73	.81	1.74
Sodium nitrate.....	1.46	.057	1.57	1.79	.024	1.43	1.98	.02	1.36
Sodium nitrate..... Calcium carbonate....	1.89	.051	.84	1.61	.017	1.45	.59	.....	3.00
No fertilizer.....	.71	.236	1.18	.75	.022	1.24	.30	.....	1.20

[a] A=beaverdam soil and sand; B=Medford loam; C=antelope-clay-adobe.

TABLE VIII.—*Weight of rape and its percentage of sulphur and nitrogen on the different soils and sand cultures*—Continued.

Treatment.	Sand.[a]					
	Extract A.			Extract B.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*		
Calcium sulphate Sodium nitrate	0.88	1.13	3.53	0.73	0.90	3.75
Sodium sulphate Sodium nitrate	1.03	.93	2.35	.62	1.40	3.38
Sulphur Sodium nitrate Calcium carbonate	.50	1.29	8.29	.35	1.51	5.58
Sodium nitrate	.59	.07	4.31	.25	.....	4.00
Sodium nitrate Calcium carbonate	.....	.....	.....	.....	.....	.....
No fertilizer	.....	.....	.....	.....	.....	.....

[a] A=beaverdam soil and sand; B=Medford loam; C=antelope-clay-adobe.

Petersen (*19*) in an analysis showing different forms of sulphur in plants found more organic sulphur in clover, rape, and radish where sulphur was present in the fertilizers used, and Ames and Boltz (*1*) report increase of organic sulphur in rape where sulphates were applied to the soil. These results and the increase in nitrogen content support the idea that maintaining a sufficient supply of sulphate sulphur and available nitrogen in the soil would tend toward more protein or other sulphur organic-compounds being formed in the plant. The sulphur content is generally increased wherever sulphur fertilizer is added. The sulphate radical is in combination with some other radical and the question arises whether the mineral content or ash of the plant is not increased by this noticeable increase of sulphate sulphur. If sulphur is applied as sodium sulphate will the sodium content of the plant be increased or if calcium sulphate is used will the calcium be absorbed by the plant?

TABLE IX.—*Percentage of total sulphur, sulphate sulphur, organic sulphur, and total nitrogen in alfalfa grown on sulphured and unsulphured portions of three different Oregon soils*

Soil.	Treatment.	Total sulphur.	Sulphate sulphur.	Organic sulphur.	Total nitrogen.
1...	Sulphur	0.227	0.0603	0.167	2.51
	No sulphur	.127	None.	.127	2.22
2...	Sulphur	.167	.0356	.131	2.16
	No sulphur	.118	None.	.118	2.01
3...	Sulphur	.200	.059	.141	2.38
	No sulphur	.118	None.	.118	2.09

Soil B responds to sulphur treatment in these pot tests while in the field elemental sulphur caused no increase in production. In the analysis of the soils in Table I soil C contains calcium carbonate, while B does not. It may be that the sulphur was oxidized in the field as in these pot tests, but as no base was present to combine with the sulphuric acid, the latter interfered with the growth. In C, calcium carbonate was present which neutralized the acidity and provided sulphates which produced the beneficial effects. Data in Table X show the difference in sulphate content between the soils receiving calcium carbonate and sodium nitrate compared to those receiving the above named compounds plus sulphur. The results show that the elemental sulphur was oxidized to the sulphate form. Furthermore, no weighable quantities of barium sulphate were obtained from the water extracts of the unsulphured soils, showing a deficiency of sulphate sulphur for immediate plant use. The rate of sulphofication appears to be greater in the beaverdam soil than in the other soils containing less organic material. While soil A has a high sulphur content and also readily oxidizes elemental sulphur, it gave a noticeable response to sulphate teatment when clover was grown.

TABLE X.—*Sulphur as sulphate in the water extract from 40 gm. of soil A and 80 gm. each of B and C after growth of plants*

[Weight in milligrams]

Treatment.	Soil A.[a]			Soil B.[a]		Soil C.[a]	
	Clover.	Oats.	Rape.	Clover.	Oats.	Clover.	Oats.
Sulphur.	21.9	34.4	25.9	4.1	10.8	10.3	11.2
Calcium carbonate. . .							
Sodium nitrate.							
Calcium carbonate. . . .	None.	None.	None.	None.	None.	None.	None.
Sodium nitrate.							

[a] Soil A=beaverdam; soil B=Medford loam; soil C=antelope-clay-adobe.

It is realized that the experiments conducted here have not been on a large scale and the conditions are not comparable to those in the field. No general conclusions can be made, but what conclusions are drawn apply only to the limits of this experiment and based upon conditions of this work where each individual case can be compared to the other. This work will be repeated on a larger scale and expanded so as to answer some of the questions which have arisen during this experiment.

SUMMARY

1. Pot experiments to show the effect of sulphur fertilizers—namely, sodium sulphate, calcium sulphate and sulphur on red clover, rape, and oats were carried with three different soils, including one with a high sulphur content, one that did not respond to elemental sulphur in the field, and one that did.

2. To eliminate the sulphur compounds acting upon the insoluble plant food and soil organisms, these plants were also grown on sand receiving the sterilized soil extract and certain pots received the additional sulphur fertilizers as the soil.

3. Sodium sulphate and calcium sulphate were added daily in solution form. The elemental sulphur was mixed with the soil and calcium carbonate at the time of sowing the seed.

4. Sodium nitrate solution was added daily to eliminate available nitrogen as a limiting factor of growth and also to determine what effect sulphates would have on nitrogen assimilation by the plant.

5. The plants were grown for two and one-half months and the dry weights of the tops were recorded. The total sulphur and nitrogen was determined in the majority of cases.

CONCLUSIONS

1. Addition of sulphate and elemental sulphur enhanced the growth of the plants grown in pots in the greenhouse.

2. The corresponding increases obtained on the soil extracts indicated that sulphur acted directly in promoting this growth.

3. The great increase in the nitrogen content of the clover grown on the soil where sulphates had been added is the result in all probability of the sulphates stimulating the action of the legume bacteria.

4. Sulphates caused increased root development and number of nodules on the clover grown in the soil pots.

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PLATE 9

A.—Clover on soil A. The top row, reading from left to right, shows the soil pots which received the following fertilizers:

Pot 1, calcium sulphate, sodium nitrate; pot 2, sodium sulphate, sodium nitrate; pot 3, sulphur, sodium nitrate, calcium carbonate; pot 4, sodium nitrate; pot 5, sodium nitrate, calcium carbonate; pot 6, no fertilizer.

The lower row, reading from left to right, shows the sand pots which received the following fertilizers:

Pot 7, calcium sulphate, sodium nitrate; pot 8, sodium sulphate, sodium nitrate; pot 9, sulphur, calcium carbonate, sodium nitrate; pot 10, sodium nitrate.

B.—Clover on soil B. The top row, reading from left to right, shows the soil pots which received the same fertilizers as in series A above. The lower row, reading from left to right, shows the sand pots which received the same fertilizers as in series A.

C.—Clover on soil C. The top row, reading from left to right, shows the soil pots which received the same fertilizers as in series A. The lower row, reading from left to right, shows the sand pots which received the same fertilizers as in series A.

A
1
2
3
4
5
6
7
8
9
10
B
1
2
3
4
5
6
7
8
9
10
C
1
2
3
4
5
6
7
8
9
10

1
2
3
4
5
6
7
8
9
10
A
B
C

PLATE 10

A.—Rape on soil A. The top row, reading from left to right, shows the soil pots which received the same fertilizers as in Plate 9, series A. The lower row, reading from left to right, shows the sand pots which received the same fertilizers as in pots in Plate 9, series A.

B.—Rape on soil B. The top row, reading from left to right, shows the sand pots which received the same fertilizers as in pots in Plate 9, series A.

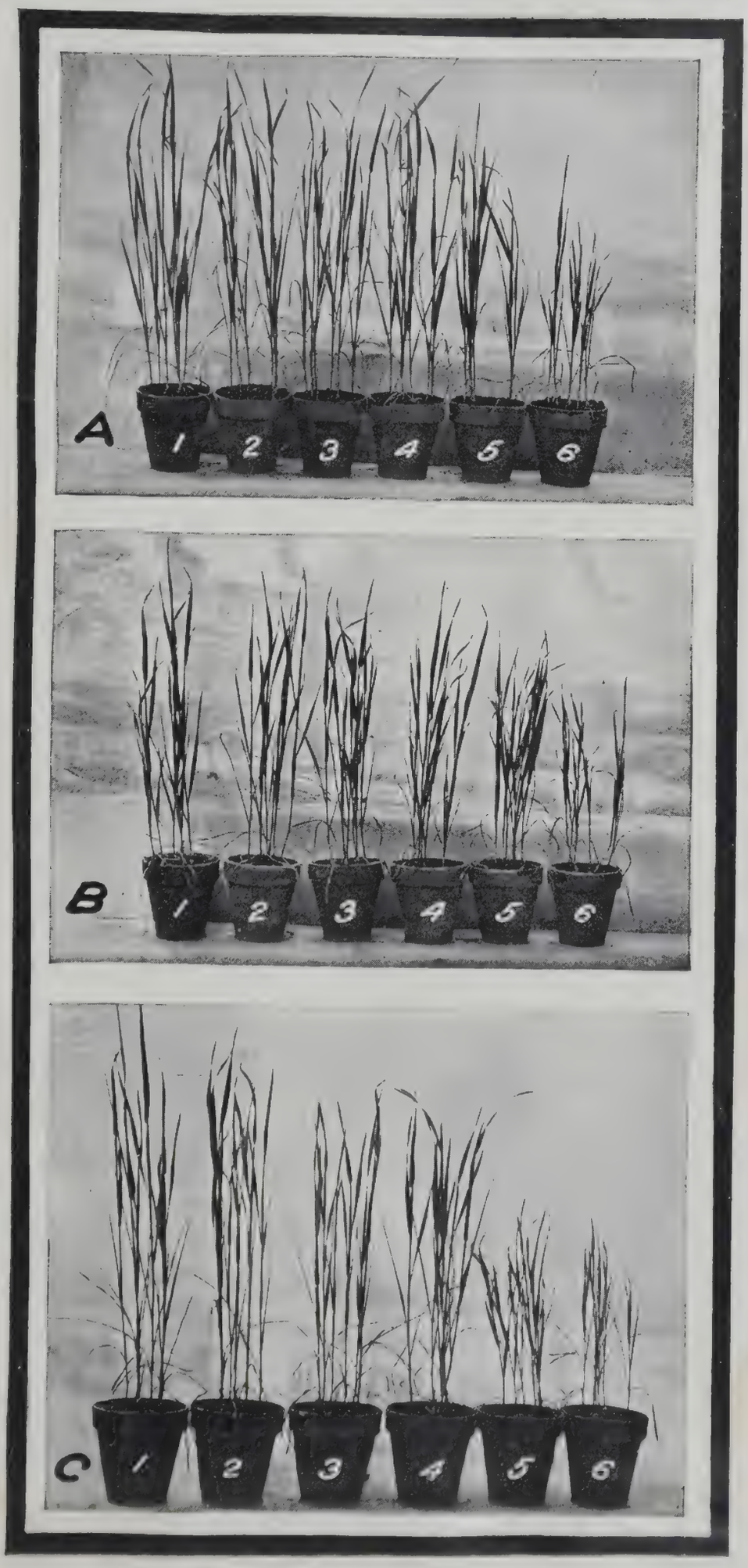
C.—Rape on soil C. The soil pots, reading from left to right, received the same fertilizers as in pots in Plate 9, series A.

PLATE 11

A.—Oats on soil A. The soil pots received the same fertilizers as in pots shown in Plate 9, series A.

B.—Oats on soil B. The soil pots received the same fertilizers as in pots shown in Plate 9, series A.

C.—Oats on soil C. The soil pots received the same fertilizers as in pots shown in Plate 9, series A.


A
1
2
3
4
5
6
B
1
2
3
4
5
6
C
1
2
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6

A
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8
9
10
B
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10
C
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8
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10

PLATE 12

A.—Oats on sand cultures from soil A. The sand pots received the same fertilizers as in pots shown in Plate 9, series A.

B.—Oats on sand cultures from soil B. The sand pots received the same fertilizers as in pots shown in Plate 9, series A.

C.—Oats on sand cultures from soil C. The sand pots received the same fertilizers as in pots shown in Plate 9, series A.

RELATION OF WEATHER TO FRUITFULNESS IN THE PLUM[1]

By M. J. Dorsey

Head of Section of Fruit Breeding, Agricultural Experiment Station of the University of Minnesota

Under suitable growing conditions the plum tree is remarkable for the uniformity with which it annually produces a crop of flower buds. Bearing a full crop of flower buds annually, however, does not insure a full crop of fruit annually; therefore, it is evident that a considerable number of flowers fail to set fruit.[2] From the standpoint of fruit production, thinning, up to three-fourths of the bloom, is actually beneficial, but beyond this the margin is approached where the thinning process reduces the yield and there is economic loss. The status of setting in controlled crosses known to be fertile under tents was similar to that in the orchard generally. This general condition led to an attempt to isolate those factors of the weather influencing the setting of fruit which result in such great differences as a complete crop failure one year and an overproduction of fruit another.

The elements of what is commonly known as "weather" which have a bearing upon pollination and fertilization are wind, temperature, sunshine, and rain. The combinations of these most favorable to the setting of fruit are sunshine, a relatively high temperature, slight or no wind, and an absence of rain. It is apparent that certain weather conditions, good and bad, go together, but temperature and rain are undoubtedly the most important elements considered from the standpoint of the setting of fruit and will be given greatest emphasis.

The following statements may be regarded as fairly typical of the conception of the influence of unfavorable conditions at bloom. Cold weather, rain, poor locality, and severe cold winter weather are mentioned by Goff (*4*) [3] as inhibiting fruitfulness. Bad weather at flowering time has an "injurious influence on fruitage" by keeping away insect visitors and affecting the fecundation of the flowers (*15*). Damage to flowers by wind, hail, rain, insects, and fungi are commonly mentioned. Lord (*11*) states that all varieties when in bloom are extremely sensitive to cold or wet weather. Heideman (*9*) notes that ample cross-fertiliza-

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[2] "Setting of fruit" is a term in common use among fruit growers. In general, it is used in referring to the number of pistils which are swelling or "setting" six weeks or so after bloom. A distinction is made in common usage between the percentage of fruit to set and the percentage of a crop, in that the latter is used in speaking of mature fruit.

[3] Reference is made by number (*italic*) to "Literature cited," p. 125–126.

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tion does not prevent great differences in the crop from year to year. Some growers hold that there is a good fruit crop only during seasons with favorable weather for bees at blooming time. Hedrick (7) analyzed the weather records of New York with respect to fruit production and showed that in general unfavorable weather is the dominant factor in crop failures. In fact, for a long time fruit growers have recognized certain weather combinations as detrimental to or prohibiting the setting of fruit.

If weather is to be assigned such an important rôle in relation to fruit fulness, the question arises as to the significance of the great variation in the time of bloom from year to year. For instance, plums have varied nearly one month in the time of flowering at the Fruit-Breeding Farm in the last seven years, the earliest bloom in this period beginning April 24, 1915, and the latest May 20, 1916. The cause for such a variation in time of bloom should not be assigned entirely to the weather of early spring, because Sandsten (*13*) found, upon analyzing the blooming records at Madison, Wis., that the time of flowering was influenced more by the growing conditions of the preceding summer and fall than by those of the spring. In Plate 15 the prevailing weather of early spring when plums are in flower is presented in some detail. It will be seen from the analysis presented in these graphs that cool weather and frequent rains can be expected in Minnesota for a period of even greater length than that covered by the greatest extremes in the time of bloom. Therefore, inasmuch as a range in blooming time of as much as one month has not meant an escape from periods of unfavorable weather, early or late blooming does not necessarily have a constant relation to fruitfulness.

The period of 10 days after bloom was selected (Pl. 15) because it covers for the most part the time of fertilization. In only 10 instances out of 142 did the minimum temperature occur in the day and the maximum at night, so that the curve of maximum temperature may be considered as the day temperature and that of the minimum as the night temperature. In the graph for each season the period of bloom is indicated by the lighter shaded portion between the maximum and minimum temperature curves. In the case of wind and the character of the day (sunshine or cloudiness) a 12-hour day was taken because of the bearing of wind and sunshine on bee flight. The date in the graph is located in the midpoint, which is 12 m. The short, broken-line curves indicate the wind velocity during the daytime only, i. e., from 6 a. m. to 6 p. m. The legend is at the right of the graph. The character of the day is shown by the shading at the base of each graph; the dark bar represents the portion of the day which was cloudy, the cross bar that which was partly cloudy, and the white the time of sunshine. A dotted line is drawn through each graph at the 40° and 51° F. points, the former

being the point Goff (*5*) found that plum pollen did not germinate and the latter the temperature of slow tube growth.

Since the weather at the Fruit-Breeding Farm has not been recorded, this analysis is made from the records furnished by Mr. U. G. Purssell, of the United States Weather Bureau, at Minneapolis.

EFFECT OF UNFAVORABLE WEATHER ON THE SETTING OF FRUIT

It has been a matter of common observation among fruit growers that when the blooming period is accompanied by a prolonged rain there is generally a light setting of fruit. Halsted (*6*), in an attempt to determine the cause of this, performed an experiment in which an apple tree was kept wet with a spray of water for six days while in bloom. The weather was fair during the experiment. The sprayed tree failed to set any fruit, except in a few instances on the upper branches, while the surrounding trees of the same variety set full.

Beach and Fairchild (*3*) performed a similar experiment with a Mount Vernon pear tree and a Duchess grapevine. The pear tree subjected to a spray for nine days bore a single fruit. Pollen taken from "fresh anthers" on the fifth day and placed in a sugar solution proved to be "perfectly capable" of germination. Many of the stigmas examined 24 hours after the experiment began were found to be "dusted with pollen," although no insects had been seen near the tree. After the close of the experiment many anthers opened and shed an abundance of pollen.

In the case of the Duchess grape, although the 12 days' treatment did not cover the entire period of bloom, the treated vines bore many aborted berries, but on none of the clusters were all of the berries aborted. Also, the average size of the fruit was reduced approximately one-half.

In these experiments the conditions which generally accompany a prolonged rain were not duplicated exactly, and consequently other factors may have entered into the results obtained. However, a constant spray was effective in preventing fruitfulness in the apple and pear, and even in the case of the grape sufficient pollination to account for the setting of fruit which took place may have occurred after the water was turned off.

It will be of interest here, after a review of the experiments of Halsted (*6*) and of Beach and Fairchild (*3*), to include a statement concerning the percentage of fruit to set in a plot of Surprise seedlings at the University Farm in order to show the general effect of unfavorable weather. All trees bloomed heavily during the seasons of 1917 and 1918 and for this reason present an excellent illustration of the effect of weather upon the setting of fruit. These seedlings are about 13 years old, fairly uniform in size, and are growing under clean cultivation. It would appear that ample pollination would take place if the weather were favorable, because these seedlings are located within less than a quarter of a mile of the University apiary of about 100 colonies. In general it may be

stated that during both seasons conditions were unfavorable for insect flight. The weather conditions at time of blooming for these two seasons are shown in Plate 15.

TABLE I.—*Comparison of fruit setting in an orchard of 226 Surprise seedlings during the two relatively unfavorable seasons of 1917 and 1918* [1]

Range in percentage of fruit to set in 1917.	Range in percentage of fruit to set in 1918.						Total number of trees.
	0	1	5	10	20	30	
	Number of trees.	*Number of trees.*	*Number of trees.*	*Number of trees.*	*Number of trees.*	*Number of trees.*	
0	3	1	.....	.....	.....	.....	4
1	5	8	.....	4	3	.....	20
5	6	9	11	14	6	4	50
10	4	13	6	9	.....	3	35
20	17	22	12	13	14	.....	78
30	12	9	2	3	3	3	32
40	3	3	.....	.....	.....	1	7
Total number of trees.....	50	65	31	43	26	11	226

[1] The percentage set is based upon the total number of flowers borne. Each tree is placed in the table with reference to the percentage of fruit set in 1917 compared with that in 1918. For instance, in 1917 there were 78 trees in which 20 per cent of the flowers set, but in 1918 the set on these same trees ranged from 0 to 20 per cent.

The data are presented in the form of a correlation table in order to show the influence of heavy fruiting during one year upon the crop the succeeding year. Accordingly, each tree is placed in the table with reference to the percentage of fruit set in 1917 compared with that set in 1918.

Three things are outstanding in Table I: (1) The heavy setting or bearing of 1917 was shown to have no distinct influence on the succeeding crop in 1918; (2) there was a heavier setting in 1917 than in 1918, the relative number of trees setting below 20 per cent being 109 and 189, respectively; and (3) since by actual count it was determined in the 6-weeks period after blooming that only one pistil in four set or persisted on those trees bearing what was arbitrarily regarded as a "full set," it will be seen that many of the trees set an unusually small number of fruits, too few, in fact, to produce a full crop after allowing for subsequent loss. This condition is not unusual in the plum when blooming time is accompanied by unfavorable weather. The light set in those trees which produced normal flowers in abundance presents a condition quite similar to that which prevailed both seasons in a number of standard varieties and other seedlings under cultivation. In Plate 13, A and B, the contrast between the number of flowers borne and the fruit to set is shown.

ANALYSIS OF WEATHER AT BLOOMING TIME

With weather apparently having such an important bearing upon the setting of fruit, as is indicated in the spraying experiment and in Table I, a more detailed analysis of weather has been made during blooming time and for 10 days after, with the object of determining whether there are certain conditions each season which can be singled out as prohibiting a set of fruit. At the outset it should be stated that there are factors which operate beyond the 20-day period to reduce the crop. Nevertheless, there are influences entering during blooming time which do not operate in the same manner anywhere else in the life cycle. As a result of the sum total of these influences a sufficient number of pistils have or have not set, as the case may be, at the 5- or 6-week period to determine definitely the prospect of a crop.

WIND

The experiments of Waugh (*16*) show that no fruit set from wind-carried pollen when insects were excluded by a covering of coarse mosquito netting. Further tests (*18*) with microscopic slides covered with vaseline, to which pollen adheres, showed that when the slides were placed at various heights and distances from trees in full bloom on bright sunny days even a direct wind did not carry sufficient pollen to bring about effective pollination at a distance equal to that from one tree to another. Wind pollination, therefore, may be regarded as insufficient, even under the most favorable conditions.

Pollination under orchard conditions is affected by windy weather, however, especially when prolonged, if insect visits are prevented. During a strong wind, rain, cold, or cloudy weather, conditions are such that insect activity is reduced to a minimum. Waugh (*16*, *17*) shows that honey bees, of the 30 or more species of insects found to visit the plum, are (*16*, p. 247) "nearly always the most active workers, and the ones which, by the character of their operations in the flower, may be held chiefly responsible for the proper distribution of pollen." These results are confirmed by Backhouse (*1*). Wind, therefore, may be regarded as having more of an indirect than direct bearing upon the setting of fruit, since pollen is not wind-carried in quantities sufficient for ample pollination. The influence upon bee flight, however, may be serious at certain times.

The curve for wind in Plate 15 runs through the point of hourly wind movement from 6 a. m. to 6 p. m. While the average wind movement considered aside from sunshine and the character of the day is of little significance, it shows what may be expected at this time of year in Minnesota. The average wind movement per hour, within the above limits, for 7 years was approximately 15 miles, while the average of the extreme wind movement recorded, within the same limits, for the 7-year period was near 19. The extreme movement recorded was 38 miles. Assuming that a wind of 25 miles per hour approaches a condition where bee

flight is hindered, it will be seen from Plate 15 that wind alone is not generally prohibitive of bee blight, but that at certain critical times, as on April 28 and 29, 1915, following a period of cloudy weather with frequent rains, it may become important—more so, in fact, from the standpoint of insect flight than from that of mechanical injury to flowers.

In addition to the considerations noted above, wind has a general drying effect upon the flower parts. Dehiscence is quickened and petals drop earlier. There is, however, no marked drying noticeable in the stigma during early receptiveness, but late in the receptive period stigmas can be found which appear distinctly dry even before the stigmatic cells are dead. Since the absorption of stigmatic fluid is no doubt the first act in germination the drying effect of wind upon stigmas may be regarded as much more critical late in receptiveness than earlier, especially in view of the more unfavorable conditions for tube growth, if pollination has been delayed.

TEMPERATURE

Temperature is primarily of interest in this connection from three standpoints: (1) Its direct effect upon pollen or pistil, (2) its influence upon pollen-tube growth, and (3) its interference with bee flight. From Plate 15 it will be seen that there are many periods of low temperature during blooming time which are occasionally accompanied by frost. With reference to direct injury, it will be interesting to record here the damage to flowers at two distinct stages of growth.

On the night of April 19, 1918, a freeze occurred at the Fruit-Breeding Farm, when the petals were just showing in the earliest blooming varieties. There was no injury to pollen or pistil, but as many as one-half of the petals were killed on some of the varieties. These bloomed, however, at the usual time, and the small dead petals persisted, while those not killed underwent the usual enlargement.

This freeze was followed by another on May 12, one week after blooming, when the flowers were further advanced. But this time all stigmas were dead on the varieties which had bloomed earlier. The calyx tube was still persistent, as there had not as yet been sufficient pistil growth to break it except in two varieties of *Prunus nigra*. Although generally there was little injury to pistils at this stage, different varieties showed considerable differences in the degree of injury. On Stella, growing in a low location, approximately 65 per cent of the pistils were killed, and on Minnesota No. 21 (Burbank × Wolf), adjacent, there was less than 1 per cent. Where injury occurred the entire pistil was killed, and in two days it turned black, dried rapidly, and dropped a few days later at the pedicel base. On the higher locations there was no injury to any of the varieties. Compared with the region in Utah in which Ballantyne (2) studied frost injury, frosts do not appear to bear such a vital relation to fruitfulness in Minnesota.

Pollen taken from flowers in which the pistils were killed appeared normal in color and in content when observed in a mount of lactic acid. Its viability, however, was not tested, but judging from appearances this freeze injured pollen much less than pistils.

Goff (*5*) shows that plum pollen was not destroyed by a short exposure to freezing temperatures. Sandsten (*14*) tested this point further and found that when plum pollen was exposed to a temperature of 29.3° F., 56 per cent germinated, compared with 62 per cent in the check, which was not exposed to the freezing temperature. He also found that the time required for germination was increased one-half as a result of the influence of the low temperature. On the other hand, 21 plum pistils exposed to the same temperature for six hours were all killed except two.

The action of low temperatures in retarding pollen-tube growth is no doubt one of the primary factors in the failure of fruit to set. The experiments of Goff (*5*) show that plum pollen does not germinate at temperatures below 40° F., and even at temperatures as high as 51° F. that there is slow pollen-tube growth. A dotted line is drawn through the graph for each year in Plate 15 at these two points. The extent to which the curve for the minimum temperature extends below the line where pollen-tube growth does not take place shows that in some seasons, as in 1915, a prolonged cool period following blooming may be the principal cause of the failure of fruit to set.

With reference to the influence of temperature upon insect flight, it appears that a definite point can not be selected below which activity ceases. Furthermore, temperature can not be considered separate from wind, rain, and sunshine. Recent investigations upon the honey bee, which is the chief pollinizer of the plum, however, show something of its response to temperature. Phillips (*12*) states that 57° F. is "the lowest temperature which normal bees ever experience in the hive." At air temperatures below this immediately surrounding the bees in cold weather, they begin to cluster. Kenoyer (*10*) in reporting the data collected over a 29-year period by J. L. Strong at Clarinda, Iowa, shows that only 1 per cent of the total honey produced for that period was collected when the temperature was below 70° F. compared with 53 per cent when the temperature ranged between 80° and 90° F. Nevertheless, this does not deal directly with the point as to what temperature prevents the pollinating activity of bees on plums in early spring.

The opinions of two bee men regarding the lower temperature in which bees will fly are as follows:

> The normal temperature for bees to take flight is 46° F. This temperature is 1 degree to 2 degrees lower for Carniolan races and up to 3 degrees lower after long confinement.[1] The individual bee can continue muscular movement only so long as the temperature of the body does not fall below 45° F., but at this temperature it loses its power of movement. (*12*, p. 59.) In general bees will not

[1] Letter from Prof. Frances Jaeger, University Farm, Dec. 31, 1918.

fly from the hive until the temperature is about 60° F. unless they are inpelled to fly by a long period of confinement resulting in an accumulation of feces.[1]

The minimum temperature curves in Plate 15 show that there are only relatively short intervals when the temperature is below 50° F. It would appear that if bees were present in sufficient numbers, other conditions being suitable, ample pollination would undoubtedly take place, at even short intervals of favorable weather.

SUNSHINE

Sandsten (*14*) showed that while sunshine had a direct influence upon fertilization in the tomato, it had none in the plum. Judging from his experiments, sunshine appears to have its chief bearing in this connection upon such factors as insect flight and general plant activity, particularly nectar secretion. Kenoyer states (*10*, p. 21) that "clear days are preeminently the days for honey production." From general observation of bee activity on plum bloom, the same may be said regarding pollination. As will be seen later, however, pollen is most readily available for dissemination in dry, sunny weather when bees are most active.

The total hours of sunshine during blooming are less than might be expected. The character of the day is indicated in Plate 15 at the base of the graph for each year by the shading. For the 7-year period there has been, while plums were in bloom, an average of only 49 hours of sunshine each season, compared with an average of 56 hours of cloudiness. The minimum was reached in 1916, when there were only 27 hours of sunshine. Alone, however, the absence of sunshine does not prohibit the setting of fruit.

RAIN

On account of the nature of the processes taking place at blooming time, rain has the most immediate action of all of the factors of weather. The fact that the period of pollination is so limited in the plum makes it possible for rain to delay normal functioning to an injurious extent. Furthermore, the stigma is exposed to weather during the limited time it functions. It will be seen, therefore, that rain may influence processes which, on account of the structure of the organs concerned, must function when more or less exposed.

EFFECT OF RAIN UPON DEHISCENCE

A study of the bloom in the orchard during a heavy and prolonged rain showed that the stamens were drawn together and held in a cluster about the pistil by a large drop of water. This was typically the condition in the absence of wind and in protected locations. The added weight of water held in this way resulted in a drooping of the branches,

[1] Personal correspondence with E. F. Phillips of the U. S. Department of Agriculture, Bureau of Entomology, Dec. 26, 1918.

and a large part of the water dripping from the tree fell immediately from the stamen cluster. When the style was the same length or shorter than the stamens, the stigma was completely immersed in water. In cases where the style was considerably longer than the stamens, the stigma projected from the drop, especially in positions where the pistils pointed upward.

During the period of drying after a rain, when the water holding the stamens and pistils is partly evaporated, the anthers break up into groups, each group, however, being still held in water. Gradually, upon further drying, the groups break up, and the anthers assume their normal position in the flower.

In order to study anther action more in detail at the time of rain, a limb which had been in bloom for three days was cut from a tree during a heavy rain and brought into the laboratory, the temperature of which was about 68° F. All anthers were closed when first brought in, but some

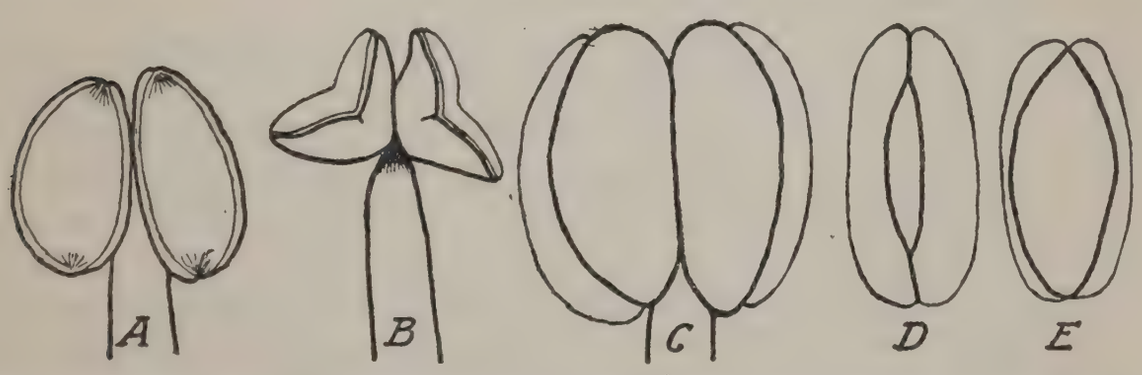

Fig. 1.—An outline drawing of an anther of Minnesota No. 12, showing the adjustment which takes place as a result of taking up or giving off water: A, an anther which has been open in the orchard for three days; B, the same with the anthers pushed up to show the dead area at the upper end of the filament; C, the appearance of the anther after two minutes in water. The anthers are completely closed and have reached their usual size; D, the degree of opening of one suture of the same anthers in 8 minutes when exposed in the laboratory at a temperature of 70° F.; E, the same anther at the end of 12 minutes' drying.

opened completely in 10 minutes under the conditions in the laboratory. When these anthers which had opened were again placed in water they closed in two to three minutes.

Furthermore, anthers which had been open for approximately 3 days and from which all of the pollen had been shed, when placed in water, closed up and in some trials swelled to the usual size in as short a time as 2 minutes (fig. 1). Other tests showed that when unopened anthers were kept in water for 2 weeks there was a slight breaking of tissue at the suture but no dehiscence. On the other hand, anthers which had once dehisced and from which the pollen had been shed closed at once when placed in water and remained closed during the 2 weeks of the test. Opened anthers held for 4 days in a saturated atmosphere under a bell jar did not absorb sufficient moisture to close them; and the experiments of Goff (5) showed that plum anthers did not open in a saturated atmosphere under a bell jar in 56 hours at a temperature of 65° to 70° F. Goff (5) also showed that in a dry atmosphere low temperatures (about 51° F.)

retarded but did not prevent anthers from opening. This shows clearly the relation of dehiscence to water.

The fact that empty anthers close during a rain and open afterwards probably has been the basis for the popular conception that rain washes pollen away.

With this statement, then, of anther action in relation to water, the question arises as to what extent rain removes pollen from anthers which have just dehisced. In investigating this point a branch of flowers was brought into the laboratory, and after the anthers opened it was stirred vigorously for 8 minutes in a pail of water. All anthers closed completely during the time of stirring. The larger part of the pollen lost occurred with the first impact with the water. After this treatment it was estimated that those anthers which were open before being put into the water still contained, when they opened again, from one-quarter to two-thirds of their pollen. These results agree with observations made in the orchard both during and after a rain.

The effect of rain in washing pollen away, even in the quantity noted above, is partly modified by the unevenness of anther opening, there being in some cases as much as 3 days' difference between the first and last opening of anthers. The unopened anthers have a light yellowish color in contrast to the water-soaked appearance of those which have been closed by rain.

These observations show that anther action is a reversible process and is controlled by water. The presence of the anther sap until the maturity of the pollen creates an internal condition unfavorable to dehiscence. If dehiscense takes place only after sufficient drying, there must be an internal control of water as well as a means for external loss. These two conditions are met by a breaking of the epidermis at the suture and by the drying or death of the cells of the filament at the point of union with the anther where there is a pronounced constriction of the filament. At this point the cells typically turn brown before dehiscence, a condition which suggests an early cutting off of water. The browning slowly extends down the filament and at the time the petals fall, 3 to 4 days after blooming, the filament is dead for a distance of 1 to 2 mm.

Under some conditions pollen is shed more quickly than under others. When anthers of Surprise were allowed to open in a dry, still room at about 72° F., at the end of four days pollen had not been shed except in very small amounts. This was due partly to the adhesive action of a yellowish, oily substance about the pollen grains which is characteristic of some varieties, and partly to the absence of wind. The persistence of pollen is further shown by specimens of Surprise grown in the greenhouse, which, at the time of abscission of the calyx tube, 10 days after blooming, still had an abundance of pollen present. But in some varieties with sticky pollen, under orchard conditions as much as one-half may still be present at the time the petals drop. On the other hand,

in some varieties of *P. americana*, pollen may almost completely disappear from the anther during a wind, undoubtedly due to drying and shaking the first day, or even the first few hours after opening. Wind pollination would be more effective in these varieties than in the others, although it is probable that it would be insufficient because plum pollen has no appendages as in *Pinus* spp. to give it greater carrying capacity.

The importance of the rapid closing of anthers upon coming in contact with water, together with the fact that they remain closed as long as they are wet, needs emphasis in this connection. It will be evident that pollination is impossible when the anthers are closed. Furthermore, the conditions which close anthers in most cases also prevent insect flight, but, even if insects were working, pollination could not take place for the reason that pollen is not available. It appears, therefore, that too much emphasis has been placed upon the action of rain in washing pollen away because anthers close quickly enough largely to prevent it.

RAIN INJURY TO PLUM POLLEN

It has been shown above that anthers take up water in sufficient quantities to close them before there is complete loss of pollen. Accompanying the drying process which takes place in the anther and the disappearance of the anther sap, there is a similar drying in the pollen. Before dispersal, pollen changes from the typical spherical shape to one distinctly oblong, and deep folds appear at the sutures. When subjected to drying immediately after removal from the anther, this change in shape takes place in 5 to 10 minutes and is quickly reversible in 3 to 5 minutes when placed in water. With these changes in mind, the question arises as to the effect of a prolonged rain upon pollen.

The rainy period at blooming time in 1915 started with a trace on April 24 and ended with rain all day on April 26 and 27. The heaviest rain, accompanied by a moderate wind, fell on April 26. During the period of the rain there was a relatively high temperature ranging from 58° to 62° F.

Following the usual cytological procedure, before drying, there were fixed in Flemming's medium anthers from 48 hybrids and varieties after the rain of April 26 and from 30 others after the rain of April 27. In all, pollen was collected from 63 crosses and 13 varieties, representing 6 species, namely, *Prunus americana*, *P. Besseyi*, *P. nigra*, *P. triflora*, *P. pissardi*, and *P. cerasus*.

It would appear that this material would furnish conclusive evidence as to whether or not plum pollen is burst by rain, as is held by Hedrick (*8*) and generally by fruit growers. A careful examination of mounted sections from each lot fixed as mentioned above, showed (1) that the pollen was not burst and had every appearance of being normal; (2) that only an occasional anther was devoid of pollen, although most of the sutures were broken; and (*3*) there was no apparent difference in the pollen condition of the different species.

EFFECT OF WATER UPON THE VIABILITY OF PLUM POLLEN

The effect of water on the viability of plum pollen was tested in the sand cherry (*P. Besseyi*). The results are presented in Table II. The time of soaking, 10 minutes, while relatively short, was decided upon because it was thought that if water was injurious at all, it would be desirable to test its effect at the shorter exposure. The time of soaking, however, is much shorter than the actual time the pollen was subjected to water, since it required some time to dry. Sixteen hours elapsed before this pollen was applied to the stigma. It will be seen from these results that soaking pollen of this species in water and drying before using has no injurious effect.

TABLE II.—*Viability test of Sand Cherry (P. Besseyi) pollen after being soaked 10 minutes in water and then allowed to dry for 16 hours, the pollen in one series having been taken from unopened anthers and allowed to dry in the sun and in the other series from open anthers and allowed to dry in the shade*

Cross made and pollen treatment.	Condition of anthers.	Number of flowers on May 25.	Number swelling on June 15.	Number set on June 26.
Treated:				
Tree No. 1×, pollen, soaked 10 minutes	Unopened	9	6	4
Tree No. 1×, pollen, soaked 10 minutes	...do	15	6	6
Tree No. 2×, pollen, soaked 10 minutes	...do	31	20	19
Tree No. 2×, pollen, soaked 10 minutes	...do	26	9	9
Tree No. 3×, pollen, soaked 10 minutes	...do	12	.....	4
Tree No. 1×, pollen, soaked 10 minutes	Opened	31	10	9
Tree No. 3×, pollen, soaked 10 minutes	...do	28	9	7
Checks:				
Tree No. 1×, pollen, not soaked	.....	7	4	4
Tree No. 2×, pollen, not soaked	.....	19	12	11
Tree No. 1×2, pollen, not treated	.....	.....	21	18
Tree No. 2×1, pollen, not treated	.....	25	11	11
Tree No. 3×4, pollen, not treated	.....	.....	.....	13
Tree No. 3×5, pollen, not treated	.....	.....	.....	11
Tree No. 4×3, pollen, not treated	.....	.....	6	3
Tree No, 5×3, pollen, not treated	.....	.....	6	1

In addition to this, germination tests were made with selected varieties to determine the effect of the rain of April 26 and 27 upon the viability of pollen. Pollen was taken from anthers which had been closed by the rain and placed in a hanging drop of 20 per cent cane-sugar solution. There was no germination even in the checks from tented trees or from unopened anthers subjected to rain. The temperature, however, which was very changeable, was quite low a good part of the time, especially at night, and the negative results with the check make it impossible to draw conclusions as to rain injury to pollen under orchard conditions.

It has been shown that on account of anther adjustment less pollen is actually washed away than has been supposed. Also, considerable quantities of pollen may be retained by anthers which have opened

immediately preceding a rain, owing to the rapidity with which they close. Anthers open as a result of drying, a condition which is brought about by cutting off the water supply at the constriction of the filament, and by evaporation, particularly from the suture. Anthers which have dehisced close quickly when brought in contact with water, and, like those which have not dehisced, remain closed as long as wet. Consequently, pollen is not available for dissemination during a rain. A careful distinction must be made between the normal shedding of pollen, which takes place for the most part the first day or even the first few hours an anther is open, and the washing away of pollen by rain, for the reason that empty anthers close when wet but open again after a rain when dry. Insect visits are reduced to a minimum, if not prevented, under the same conditions that impede pollen dispersal. The cytological studies show that plum pollen does not burst when wet by rain and crossing tests show that it is not killed by moderate exposures to water, although the results of Sandsten (*14*) indicate that humidity decreases its longevity. As far as the pollen is concerned, therefore, a prolonged rain acts primarily to delay pollination until conditions are again restored which are favorable to dehiscence and dissemination.

THE STIGMATIC SURFACE

As in the case of anther and pollen, a study has been made of the changes of the pistil during the functional period, which may be regarded as a critical stage viewed from the standpoint of the relation of adverse weather to the setting of fruit.

Immediately before the receptive period the outer cells of the stigma are turgid (Pl. 14, C and D) and their papillate structure gives to the surface a characteristic velvety appearance which is readily distinguished from the glossy, moist surface when receptive. Where the suture terminates, the stigma has a distinct depression, and in the plum its surface is more or less oblique to the axis of the style, with the higher margin opposite the marginal suture fold.

The terminal cells are one layer thick, and in longitudinal sections are clearly distinct from the cells below on account of their large size, scant cytoplasm, and conspicuous vacuoles. There is a slight variation in the length of these cells in different species. In some, as in Sapa (*P. Besseyi* × *P. triflora*), they contain spherical bodies, which stain deeply and vary much in size, the larger ones being somewhat greater in cross section than the nucleus. The scant cytoplasm in the terminal cells is mostly located at the extreme terminal end in the form of a crescent.

THE RECEPTIVE STIGMA

Decided changes are noticeable in the terminal cells after the stigma has become receptive. In sections made from stigmas 48 hours after first becoming receptive the papillate cells are very irregular in outline

and typically are collapsed and shrunken. A few cell walls appear to be broken. The cytoplasm is much contracted and drawn out into irregular vacuolated strands. The nuclei are generally irregular in outline and show evidence of disintegration. In many of the stigmas the papillate cells are partly broken away from those beneath, and the pollen grains are found among, or even beneath, the collapsed and partly separated sheath composed of the terminal cells.

Heideman states (*9*, p. 191) that the "actual time during which fertilization may be effected scarcely exceeds two hours." Observations here show that under normal conditions the plum stigma remains receptive for a maximum period of about one week. At the end of three to five days, however, the stigma begins to turn brown, and as it becomes dead and dry at the end of the receptive period the color gradually deepens to a dark brown and then black. The dark color slowly extends down the style, which, as a rule, abscisses before turning brown more than two-thirds of the way to the abscission layer. In this way the possible time of pollen-tube growth on the stigma is limited to a relatively short period. The significance of this will be emphasized in connection with the discussion on the rate of tube growth.

THE ACTION OF RAIN UPON THE STIGMA

The prevailing belief among fruit growers is that the chief injury of rain to the stigma, aside from washing pollen from it, is the dilution of the stigmatic fluid to such an extent that the growth of the pollen tube is prevented. Immediately after a heavy rain during full bloom on May 9, 1918, a study of stigmas under orchard conditions showed that even those which were past the receptive stage, dark brown in color and partially dead, were distinctly moist and turgid.

Following these observations an investigation was made of the action of water upon the stigma. When one which had been receptive for about three days was dipped in water and carefully withdrawn, a small droplet about the size of the stigma adhered. This droplet was absorbed in approximately one minute. The dipping was repeated eight consecutive times in as many minutes, and in each case the droplet was as quickly absorbed as in the first instance. As a result of the absorption of water the papillate cells became distinctly turgid. A similar test was made with an unreceptive stigma and also one which had passed the receptive stage and of which the papillate cells had become dark brown and partially dead. The same imbibition of water took place with these two as with the receptive stigma.

It will be evident that absorption of water in such quantities acts to dilute the cell sap of the papillate cells. This, however, would appear to be of no immediate consequence, since pollen does not take up the stigmatic fluid until it is secreted, and even if pollen in this way came

in contact with water before the stigmatic fluid, this, as has been shown, would not be prohibitive of subsequent normal development. Furthermore, since tests show that germination takes place in a considerable range of concentration in a sugar solution, a partial dilution of the stigmatic fluid as a result of water absorption would probably not alone prohibit tube growth. Under greenhouse conditions and in the orchard under favorable conditions a stigma, like a leaf gland, has more than one period of active secretion. If the first fluid to be secreted was completely removed by rain, it would be again renewed under favorable conditions, so that a short rain alone would not necessarily be detrimental. Even if the secretion were considerably diluted following a rain, evaporation from the surface would result in a gradual concentration. Furthermore, the influence of rain upon the stigmatic secretion could be considered of more importance if the stigma had only a single, short period of activity.

WASHING OF POLLEN FROM THE STIGMA

The adherence of pollen to the stigma was first noticed in pistils which had gone through the washing and numerous changes of solution in the preparation for sectioning by the usual cytological procedure. Stigmas which had passed through a 2-day rain, in addition to the cytological process, still held as many as 40 to 50 pollen grains.

An examination under orchard conditions of stigmas which had been subjected to a heavy rain of over 14 hours duration, showed that most of the stigmas still retained a considerable quantity of pollen (Pl. 14, B). On one stigma 42 grains were counted. On another, which had passed through a 2-day rain while in bloom, there were 32 pollen grains, and 6 days afterward on still another there were 176. However, in the last instance a part or all of the pollen could have reached the stigma after the rain.

In order to determine how readily pollen can be washed away, an abundance of pollen was placed on a stigma which was then immersed in water, the results being observed with a binocular miscroscope. At the first impact of the water a few of the outlying grains were washed away, but at the end of 10 minutes of vigorous stirring and dipping in a pail of water, 73 grains still adhered to the stigma. While the number of grains at the start was not counted, it was estimated that less than one-fourth were lost. The outstanding fact is that not all of the pollen was removed by a washing action, certainly as vigorous if not as prolonged as a rain.

An explanation of the adhesion of pollen is found in the condition of the respective stigma. In some of the fixed preparations there is a slight staining area beyond the terminal cells of the stigma (Pl. 14, A and B), in depth about equal to the thickness of two or three pollen grains. This undoubtedly represents the area in cross section of the stigmatic

fluid. Sections of stigmas show, as mentioned above, that during the later stages of receptiveness pollen may be even partly sunken in among the terminal cells. This, together with the gelatinous or viscous nature of the stigmatic fluid, expecially some time after receptiveness, largely accounts for the difficulty in washing pollen from the stigma. Also, the inward movement of water would partly counteract the washing action, especially of light rains. In addition, during the early stages of pollen germination the tubes tend to prevent pollen from being washed away. At this time, however, the tube becomes the important consideration instead of the pollen.

All pistils are not subjected alike to rain action. Those on the upper side of limbs and in terminal positions receive the direct impact of rain, while those in the more protected positions, as in the interior parts of the tree and on the under side of clusters, are shielded from the direct force of the rain.

It will appear from the foregoing that pollen is not so completely washed away by rain as has heretofore been supposed. This belief has become general on account of the changes which take place in pollen when it is placed upon a receptive stigma. Immediately upon coming in contact with the stigmatic fluid, pollen becomes turgid and is more or less immersed in it. Under these conditions its appearance closely resembles that of the terminal cells of the stigma. If a dilution of the stigmatic fluid and the washing away of pollen are the important inhibiting factors in the setting of fruit, a short dashing rain at blooming time would, at certain stages, do as much damage as a prolonged rain, because it would be necessary for the pistil to again become receptive and pollination to again take place. This, however, does not correspond with the general observations of fruit growers nor with the conditions reported here.

LIMITATIONS UPON FERTILIZATION

If the statements regarding the effect of rain upon pollen and stigma are correct, the failure of the plum to set fruit during unfavorable weather conditions will have to be explained in another way. At the time the pollen and pistil are maturing and functioning other factors are operating which place certain definite limits upon the time fertilization is possible.

On account of self-sterility, the relative time of dehiscence and receptiveness within the variety is not an important factor in the plum. However, because the pollen is mature before the stigma and virtually in a "resting stage" protected by a thick covering in addition to the anther wall, it is less susceptible to injury than the stigma, in which growth changes are still taking place. This difference in the relative maturity of the two structures may largely account for the greater hardiness of pollen during frosts. Upon germination the pollen enters a phase of less resistance, and it shares to a greater extent the lot of the stigma and style, which constitute the substratum for the pollen tube.

The factors, then, which place a time limit upon the mutual functional period and which have a direct bearing upon the setting of fruit are (1) the longevity of the pollen, (2) the length of the receptive period and life of the stigma, (3) the abscission of the style, (4) the rate of the pollen-tube growth, and (5) the influence of low temperature upon pollen germination.

THE LONGEVITY OF PLUM POLLEN

The results of Sandsten (*14*) showed that plum pollen collected from such widely separated sources as Washington, Missouri, Tennessee, and Minnesota retained its germinating power for six months when subjected to the normal humidity and temperature changes incident to the period of the test. There was a gradual decline, however, in the percentage of germination from an average of 54 per cent at the end of the first month to about 8 per cent at the end of the sixth. Furthermore, relatively adverse conditions do not affect the longevity of the pollen, since short exposures to water do not kill it and freezing temperatures only retard germination. Under favorable conditions, therefore plum pollen retains its viability considerably longer than it is functional under orchard conditions.

LENGTH OF RECEPTIVE PERIOD AND LIFE OF THE STIGMA

As has been noted, the plum stigma is receptive under orchard conditions for a maximum of one week but begins to turn brown at the end of approximately three to five days. Adverse weather conditions may, however, extend the functional period somewhat, particularly when accompanied by low temperatures. The delay in pollination up to a certain point does not prevent tube growth. Crosses were successful in the greenhouse on stigmas which were receptive four days previous to the application of pollen. Under these conditions, however, drying and browning does not take place as quickly as in the orchard where the active period of secretion is over at the end of three to five days and is followed by a period of partial inactivity of the stigma.

Furthermore, the stigma is more easily dried by the wind late in the receptive stage than immediately after becoming receptive. Tube formation would undoubtedly be more uncertain if pollination were delayed until late in the receptive period, as would be the case during a prolonged rain. Pollen germination, as well as considerable tube growth, must, therefore, take place if fertilization is to be effected within a relatively short time and before the conditions of the stigma prohibit tube growth or before dying back in the style overtakes tubes which have been formed.

ABSCISSION OF THE STYLE

The styles do not begin to absciss until about two weeks after blooming (Pl. 14, E), although the abscission layer at the point of abscission near the

base becomes very distinct in some varieties, as Winnipeg (*P. nigra*), as early as 8 days after blooming. In this variety at the 8-day period the cells in the abscission layer had reached an advanced stage in their disintegration, and while the style was still persistent, it was much lighter in color above the point of abscission, a condition which suggests the cutting off of food material. If the pollen tube had not passed the abscission layer by this time, it is probable that it would not have done so, since it would have had to grow through a region of partly disintegrated cells. Consequently all tubes which had not passed the abscission layer by the time of the abscission of the style (Pl. 14, F) would be definitely eliminated as far as fertilization is concerned. Tube growth from the stigma to the abscission layer, therefore, must take place between the beginning of receptiveness and the shedding of the style.

If pollination occurs late in the receptive period, the condition of the stigma begins to change so rapidly that only favorable growing conditions for the tubes will enable them to pass the abscission layer before the style drops. In this way the abscission of the style sets a definite time limit to a certain minimum of tube growth which may be as short as 4 days and as long as 12. It will be clear then that the later in the receptive period pollination takes place and the more tube growth is retarded, the more uncertain fertilization becomes.

RATE OF POLLEN-TUBE GROWTH

It will be seen from the above that the rate of pollen-tube growth becomes an important factor in fertilization, especially during unfavorable weather accompanied by rain and low temperatures. In order to determine the rate pollen tubes advance down the style, this point has been studied in fixed preparations of pistils taken under orchard conditions and also from controlled crosses in the greenhouse where the time of pollination could be determined definitely. The greenhouse temperature during this experiment was not recorded, but varied from 55° to 65° F. Pistils from the orchard in all cases were collected after a period of variable weather of rain and low temperatures. The results showing the extent of tube growth under different conditions are presented in Table III.

Sandsten (*14*), in determining the time required for the pollen tube to reach the ovary, cut the pistils off controlled crosses at intervals of 48 and 60 hours, respectively. From the data he presented it appears that the plum is fertilized at the 60-hour period. It should be stated, however, that this shows that tube growth had merely extended below the point at which the style was cut in that time. The 7-day period, at which time the final observations were made, is too soon to determine certainly whether fertilization has taken place judging from size alone.

TABLE III.—*Rate of pollen-tube growth found in the plum in controlled crosses in the greenhouse and under orchard conditions*

UNDER GREENHOUSE CONDITIONS

Cross.	Time.	Tube growth in greenhouse.
Minn. No. 10 [a]×Minn. No. 12.[a]	16 hours	1/6 of style length. Cross sterile.
Minn. No. 10 [a]×*P. Besseyi*	do	1/2 of style length.
Minn. No. 12 [a]×Minn. No. 21.[a]	9:30 a. m. to 3:30 p. m.	None.
Do	17.5 hours	1/10 of style length.
Minn. No. 12 [a]×*P. Besseyi*	19 hours	1/4 of style length.
Minn. No. 21 [a]×Minn. No. 12.[a]	51 hours	Do.
Minn. No. 21 [a]×Minn. No. 10.[a]	69 hours	1/10 of style length.
Minn. No. 6 [a]×Surprise	6 days	Full style length. None fertilized.

UNDER ORCHARD CONDITIONS.

Minn. No. 21 [a]×open-pollinated.	3 days after blooming	No tube growth. Rain and frost.
Minn. No. 35 [b]×open-pollinated.	do	Do.
Do	6 days after blooming	1/4 of style length.
Minn. No. 12 [a]×open-pollinated.	do	Do.
Do	10 days after blooming	Tube in embryo sac.
Minn. No. 12,[a] selfed	4 1/2 hours	Tube just formed.
Do.[c]	24 hours	1/10 of style length.
Minn. No. 6,[a] selfed	2 days	Do.
Manitoba, selfed	8 days	1/4 of style length.
P. Besseyi, selfed	12 days	2/3 of style length. Ovule aborted.
Surprise, selfed	6 days	1/10 of style length.

[a] A cross between Burbank and Wolf. [b] A cross between Abundance and Wolf. [c] See Pl. 14, A.

From Table III it appears that pollen-tube growth is relatively slow in the plum and that the time required for the tubes to reach the ovary is much longer than Sandsten estimated. Furthermore, it should be emphasized that in the above table the maximum tube growth is given.

It will be seen in the case of Minnesota No. 21 and No. 35 that there was no tube growth three days after blooming when open-pollinated under orchard conditions. The weather conditions previous to the time stigmas were collected from these two varieties will be of interest here. Both came into bloom on May 20, 1917, which was clear, with a maximum temperature of 62° F., with a slight rain in the evening, and a medium wind the latter part of the day. At night the temperature fell and there was frost. May 21 was cloudy, with a heavy rain accompanied by a strong wind lasting from early morning up to 2 p. m. May 22 was cool and clear, and the stigmas of these two varieties were collected in the early forenoon.

The time of pollination is uncertain, but bees were present in large numbers on May 20. On a single stigma of Minnesota No. 35 there were 162 pollen grains, mostly embedded in the stigmatic fluid. There were fewer grains on the stigmas of Minnesota No. 21. In the field records, made at the time of fixing this material, it was stated that the "stigmas were brown in all cases and dead in some." From this it will be seen that the receptive period was much shorter than is common in the plum. The condition, then, in these two varieties was as follows: (1) Pollination had taken place, (2) on the third day after bloom no tubes had formed in the stigmas examined, and (3) the end of the receptive period had been reached.

On each variety the dying back in the styles averaged 5 mm. by May 31, and by June 2, 13 days after bloom, the abscission layer was fully formed and disintegration of the cells in it had started. On this date additional pistils were collected and fixed, and in these pollen tubes could not be found in the micropyle, nor had embryos formed in any of the six which were sectioned. This is not surprising when it is noted that under the favorable conditions of the greenhouse, Surprise pollen tubes required six days to grow the full length of the style.

These trees under observation were 8 years old from planting and were under clean cultivation. On Minnesota No. 21, 25 per cent of the buds were winterkilled and only 5 per cent of the flowers set fruit; on Minnesota No. 35, 10 per cent were winterkilled and the percentage of fruit to set was 10. On each there was a light crop of ripe fruit.

In the case of these two varieties, then, the small percentage of fruit to set is not necessarily due to a lack of pollination, but apparently to the delay in tube formation, during which the stigmas turned brown and some died, conditions which either prevented or delayed tube growth. According to this, in those fruits which set, tube growth had either started on the 20th, before the rain, or was sufficiently rapid after it to pass the abscission layer before the style fell. The weather conditions for this season are analyzed in Plate 15.

From Table III it will be further seen that under the favorable growing conditions of the greenhouse, the rate of tube growth is so slow that the abscission layer is passed dangerously near the time of dehiscence. In the orchard, however, during the most suitable conditions, fully as many fruits set as in the greenhouse, and it is very probable that the tube extension is even more rapid.

The bearing of low temperatures upon the status of tube growth noted above warrants further discussion. The lower temperature limit of pollen germination in the plum was determined by Goff (5), as previously noted, to be approximately 40° F. At 70° F. there was an abundance of tube growth, and at 51° F. the rate of growth was intermediate between the two extremes. Entering the factor of humidity in relation to temperature, his experiments further show that there was

greater germination after five days, when pollen was kept in saturated air in a refrigerator (the temperature is not given), than under the same conditions at room temperature. This being the case, the cooler temperatures usually accompanying prolonged rains would be more favorable to a higher percentage of germination than higher temperatures. From Plate 15 it will be seen that each season the minimum temperature falls below the lower limit of tube growth a number of times and occasionally the lower limit of tube growth is even approached by the maximum temperature. It is probable that the temperature influence on tube growth would be similar to that on tube formation.

The slow pollen-tube extension found under greenhouse conditions serves as a basis for estimating what can be expected during periods of low spring temperatures. That low temperatures have a much greater influence some seasons than others is clearly shown by the extent the minimum-temperature curve extends below the line of no tube growth (5) drawn through each graph (Plate 15) at 40° F. The temperature factor, therefore, has an important bearing upon the extent to which fertilization fails to take place some seasons. While cool weather retards tube growth, it does not appear to change materially the time of abscission of the style.

RELATION BETWEEN THE WEATHER AT BLOOMING AND THE SETTING OF FRUIT

With the foregoing analysis of weather in mind, it now remains to be seen whether there is any correlation between the weather conditions prevalent at bloom and the setting of fruit. While an ample set of fruit does not certainly insure a full crop, a full crop can not be obtained unless there is a set up to a certain point. In this way the weather determines the possibility of a crop.

During the years 1915, 1916, and 1917 there was a light set and a light crop of plums at the Fruit-Breeding Farm. An inspection of Plate 15 shows that different weather combinations occurred during each of the three years. In 1915, the outstanding features are the frequent rains during bloom and the low-temperature period for one week following. This single factor, according to the work of Goff (5) on the temperature limits of tube growth, would make fertilization uncertain, but it will be noted that following the cloudy, rainy weather of the first four days of bloom there were two days of unusually windy weather which interfered with bee flight at a critical time, and hence rendered ample pollination uncertain. The following year, 1916, bloom was nearly a month later and was accompanied by a period of unusually high temperature which extended to the period of tube growth. This alone would have been very favorable to pollination, but during early bloom there were two unusually heavy rains and five lighter ones. Moreover, aside from actual injury to the bloom during such rains as

occurred on May 21 and 25, as well as the interference with insect flight, pollination would appear to be uncertain because pollen was not available for dissemination a large part of the time. This year, therefore, it appears that pollination was uncertain instead of fertilization, as was the case the year before. At any rate, during these two seasons the temperature at bloom was very different. In 1917 rain, high winds, low temperatures, and even frost, were prevalent during bloom, and at the close of bloom there were nearly 3 days of cool, rainy weather which came at a critical time during tube growth. In addition to this, frequent rains and a relatively low temperature at the latter part of the 10-day period following bloom supplemented the retarding effect of the 3-day rainy period. The wind on May 20, 21, 22, and 26 was strong enough to interfere with the work of bees. Both pollination and fertilization were uncertain this year.

In contrast to the slight set of these three seasons there was a good set in 1912, 1913, and 1918, and a heavy set in 1914. It now remains to be seen whether there were conditions at bloom these seasons which differ markedly, as far as the influence on pollination and fertilization is concerned, from the others. In 1912 the temperature was relatively high, except for three days, during the entire period. The rains were slight at bloom. Also, in 1913 the temperature was within the range of fast tube growth a good part of the time and rains were unusually scant at bloom. The unusually high temperature in 1914 is in marked contrast to the low temperature the following year, and in the absence of heavy rains there was the greatest setting of fruit as well as the heaviest crop of all season included. The high temperature at the beginning of bloom in 1918 gradually fell toward the end and there was a frost the night of May 12. The rains were not prolonged during bloom, but the heavy rain of May 9 delayed pollination in the later blooming varieties. The warm period following bloom, however, counterbalances the cooler 4-day period at the end of bloom, so that the rate of tube growth was in general increased. The setting of fruit was sufficient for a good crop this season.

It will be seen from this brief analysis that there are conditions each season which can be correlated with the set of fruit. With a light set it is impossible to get a heavy crop. As early as the 5- or 6-week period the possibilities of a crop are determined.

SUMMARY

(1) Unfavorable weather at blooming time may completely prevent the setting of fruit in the plum, even though there be a full bloom. A study of the manner in which weather affects the processes at bloom shows that rain and low temperatures are the most important factors, although strong winds when prolonged are also important.

(2) Wind has its influence indirectly by interfering with insect action and, hence, pollination at critical times. It is seldom strong enough to cause much direct mechanical injury. The experiments of Waugh show that wind pollination is insufficient, even under the most favorable conditions. Frosts during bloom are only occasional and injure the pistil more than pollen. The greatest damage from low temperatures is in the retarding of pollen-tube growth. Other conditions being favorable, cloudiness does not prevent the setting of fruit. Rain prevents pollen dissemination by closing the anthers or by preventing them from opening, but does not burst pollen nor kill it.

(3) On account of the adhesive action between stigma and pollen, rain does not completely wash pollen from stigmas. The stigma is receptive for 4 to 6 days, and following the active period of secretion the stigmatic cells rapidly disintegrate. The style abscisses in 8 to 12 days after bloom. Tube growth appears to be relatively slow in the plum even under favorable greenhouse temperatures. As a result of the rapid disintegration in the stigma and the abscission of the style, a delay in pollination or slow tube growth when the temperature is low renders fertilization uncertain.

(4) An analysis of the prevailing weather at bloom shows that each season certain sets of conditions can be singled out as being largely responsible for the status of the setting of fruit. In one season rain during bloom may be the limiting factor and in another low temperature during the period of tube growth. Unfortunately, practical remedies under orchard conditions do not appear readily available. Late blooming has not escaped unfavorable weather, and, since tube growth seems to be the process most directly affected by low temperatures, remedial measures can most effectively be sought in suitable pollinizers which show the fastest tube growth.

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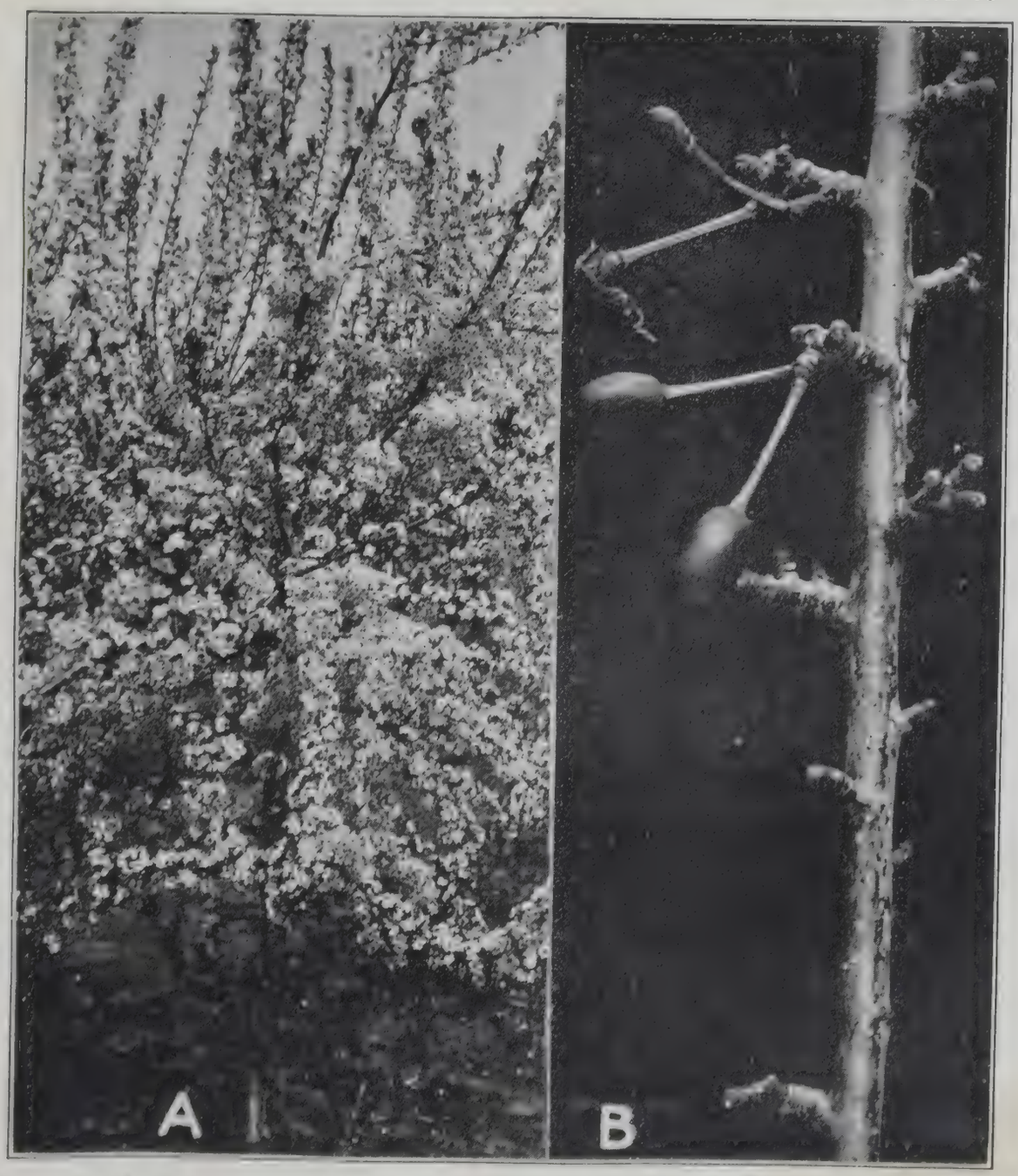
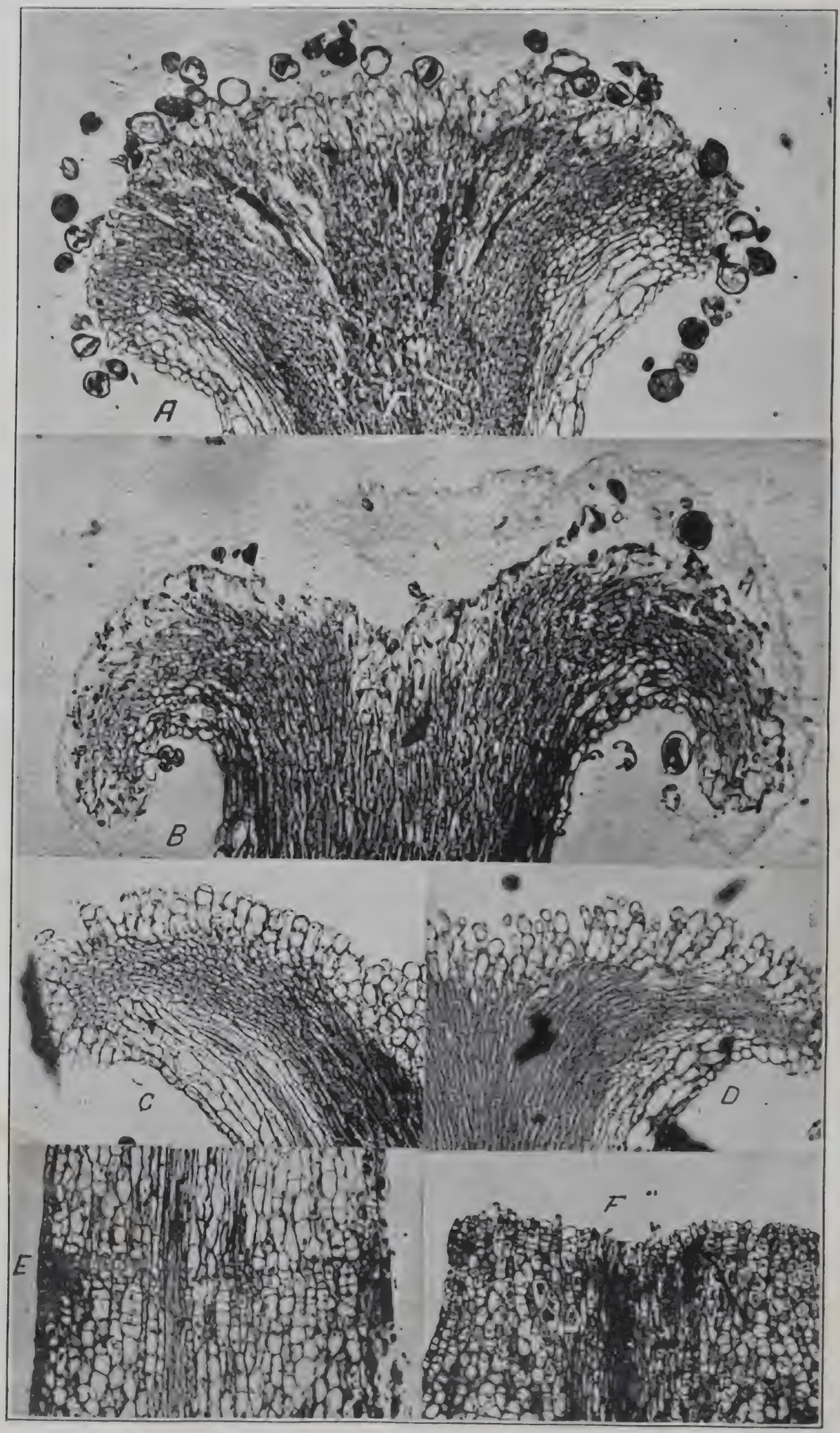
PLATE 13.

Plum tree and fruiting branch showing difference between number of flowers borne and quantity of fruit set:

A.—The appearance of a plum tree bearing a normal crop of bloom.

B.—A single fruiting branch 2 years old showing the contrast to A. Only 2 fruits have set out of approximately 100 flowers borne by this branch. Note the stubs where flowers have dehisced.


A
B


A
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D
E
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PLATE 14.

A.—Stigma of Minnesota No. 21, a greenhouse tree, 24 hours after being selfed, showing the condition of papillate cells in the stigma, pollen tubes, and also traces of the stigmatic fluid.

B.—Stigma of Minnesota No. 35, open to cross pollination, showing the condition of a stigma three days after bloom, having withstood a rain of 0.87 inch which fell in the two days previous, lasting in all 18 hours. Note the slight staining area of the stigmatic fluid in which two pollen grains are embedded.

C.—The turgid papillate cells in Sapa before receptiveness.

D.—Opata. Same as C. Pollination has not yet taken place.

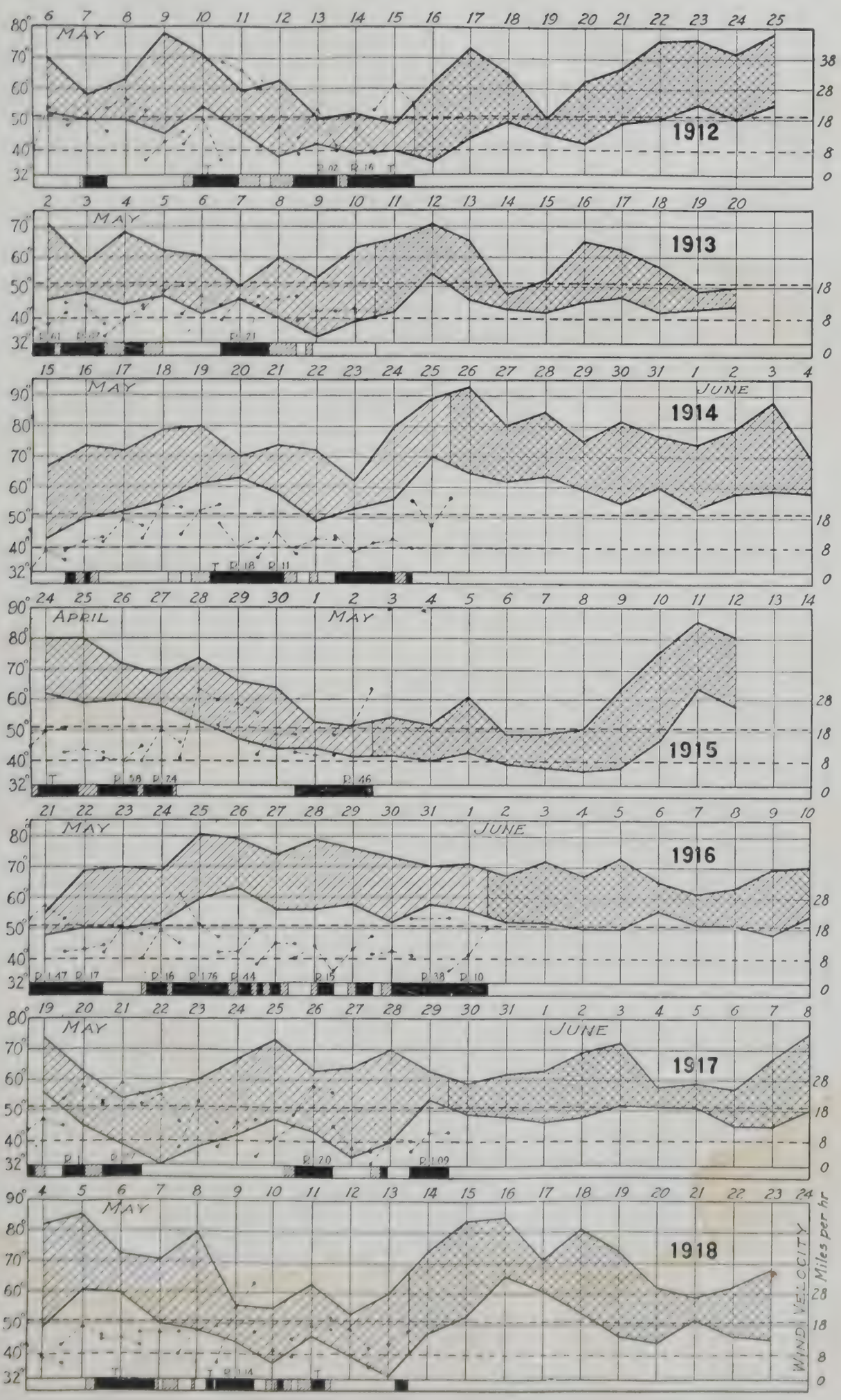
E.—Abscission layer Minnesota No. 35, showing the cells of the layer 11 days after bloom.

F.—The surface at the abscission layer of Assiniboin after the style has fallen, 12 days after bloom. There appears to be no marked disintegration of the cells immediately below the abscission layer, which suggests that in cutting off the style by this method the breaking down of the middle lamella is restricted to a few cell layers.

108123°—19——4

PLATE 15

Graphic analysis of the weather from the standpoint of wind, sunshine, rain, and temperature for seven years from 1912 to 1918. The maximum and minimum temperature range is given for each day during bloom and for a period of 10 days afterwards.


MAY
1912
MAY
1913
MAY
JUNE
1914
APRIL
MAY
1915
MAY
JUNE
1916
MAY
JUNE
1917
MAY
1918
WIND VELOCITY Miles per hr

STRUCTURE OF THE MAIZE EAR AS INDICATED IN ZEA-EUCHLAENA HYBRIDS

By G. N. Collins

Botanist, Office of Acclimatization and Adaptation of Crop Plants, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In attempting to trace the origin of maize (*Zea mays* L.) the center of interest is the evolution of the peculiar form of inflorescence, especially the pistillate inflorescence, or ear.

Since Euchlaena (*Euchlaena mexicana* Schrad.) or teosinte, the nearest known relative of maize, has a very different type of pistillate inflorescence, it may be instructive to compare the two genera and trace the successive changes that would be required in passing from the Euchlaena form of pistillate inflorescence to the maize ear.

Euchlaena and maize cross freely, resulting in intermediate hybrids which in subsequent generations grade back to the parental forms (Pl. 16). It is therefore possible to present a complete series of intermediates, graduated to any desired degree of minuteness. It should be kept in mind that although we may be able to arrange a continuous series of forms ranging from Euchlaena to maize, these forms may not represent the course of evolution. A study of these intermediate hybrids may be expected, however, to throw light on the morphology of the ear and to explain its evolution, at least in a mechanical sense.

DESCRIPTION OF MATERIAL

The forms here described as intermediate between maize and Euchlaena appeared for the most part among the descendants of a cross between Florida teosinte and a diminutive variety of popcorn called "Tom Thumb." Of this cross six first-generation plants were grown and from the self-fed seed of one of these a second generation consisting of 127 plants was produced. Several hundred third-generation plants from open pollinated seed were also examined.

Although in general appearance the pistillate inflorescences of maize and Euchlaena are so unlike that comparisons are difficult, the structure of the flowers is practically identical. The chief differences are therefore to be sought in the structure of the inflorescence and the arrangement of the spikelets.

To avoid circumlocution it is necessary to consider as a morphological unit the association represented by a sessile and pedicelled spikelet, as they occur in the staminate inflorescence. It would be misleading to refer to this unit as a pair of spikelets, because the same unit must also be kept in mind in considering the pistillate inflorescence where one of the spikelets may be suppressed. The two spikelets of a pair probably arise from a single metamer, at least they seem never to become separated. In the pistillate inflorescence, however, the individual metamers can be distinguished with difficulty and the pairs of spikelets become so profoundly and diversely modified that a general term is needed to designate this structural unit in all its forms.

In the pistillate inflorescence the members of this morphological unit, whether it is represented by one or two spikelets, occupy a single alveolus, and the complex might be described as the contents of an alveolus. In the staminate inflorescence, however, the depression in which the spikelets are borne is usually too slight to be termed an alveolus. It seems desirable, therefore, to derive the general term from some word that carries the same implication as alveolus but which has not been used in a specific morphological sense. The word *alicole* [1] is proposed and will be used in the following description to designate the spikelet or spikelets, whether staminate or pistillate, that are borne in a single alveolus or at a single point on the rachis, considered as the axil or point of attachment of a reduced branch.

The principal differences between the pistillate inflorescences of Zea and Euchlaena may now be contrasted as follows:

Euchlaena	*Zea*
Single spikelets	Paired spikelets
Two-ranked alicoles	Many-ranked alicoles
Separate alicoles	Yoked alicoles

SINGLE AND PAIRED SPIKELETS

The difference between single and paired spikelets will be best understood by considering first the arrangement of the spikelets in the staminate inflorescence of Euchlaena, which is identical with that of the lateral branches of the staminate inflorescence of maize. Since Euchlaena lacks the specialized central spike of the maize tassel it may be taken to represent the primitive arrangement of the spikelets.

In these staminate inflorescences each alicole consists of two spikelets—one sessile, the other pedicelled. The alicoles are disposed on the two sides of the branch, leaving the lower, and, to a less extent, the upper side of the branch, naked. The sessile spikelet is borne slightly below the pedicelled, that is, toward the abaxial side of the branch. Thus when viewed from the end of the branch the arrangement of the spikelets would be such that instead of an alternation between pedicelled and

[1] *Ala*, armpit+*colo*, inhabit.

sessile spikelets, the two sessile spikelets would stand next each other as would the two pedicelled spikelets (see fig. 1, A). This lack of radial symmetry will be shown to be a very persistent and important feature.

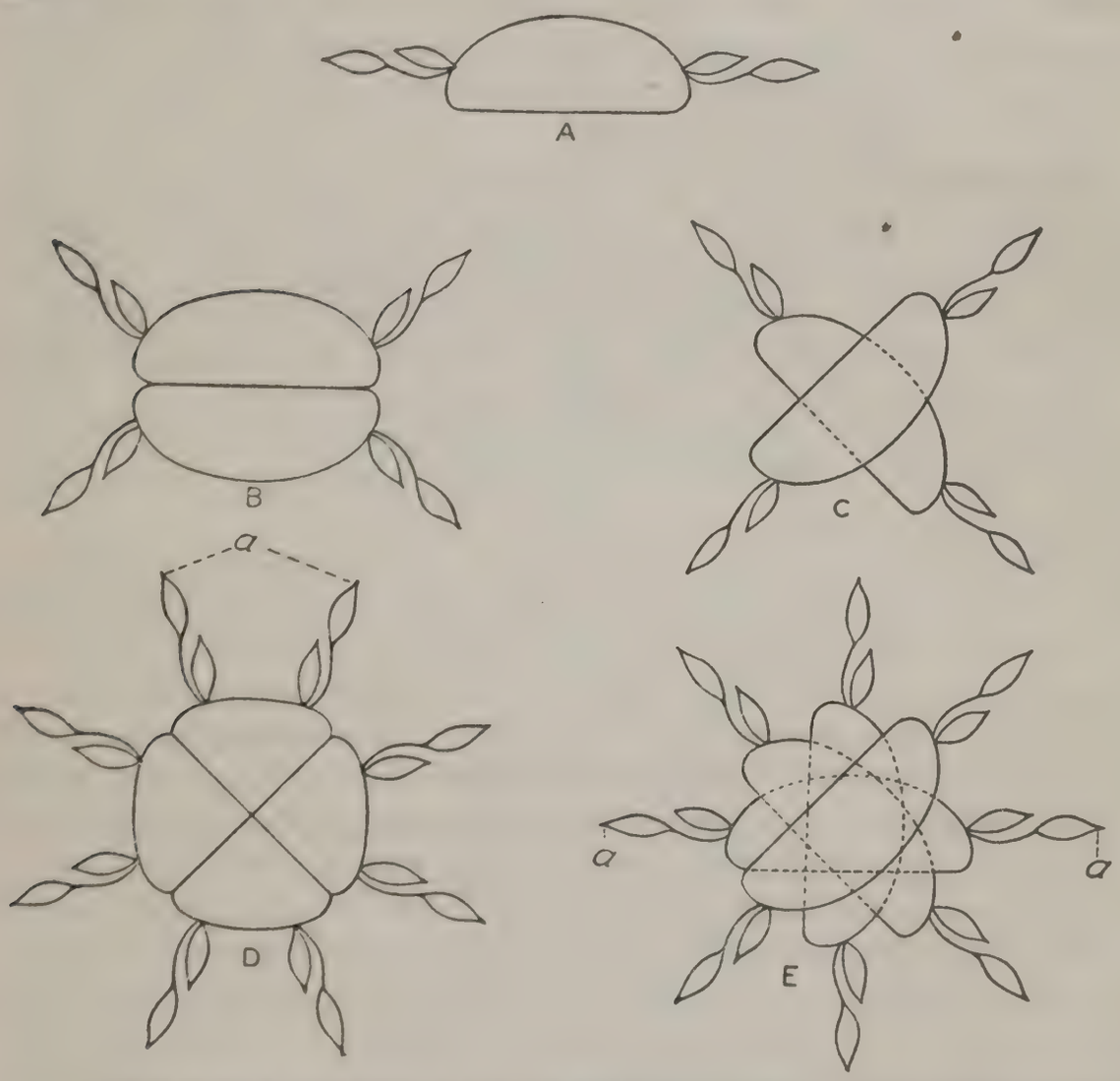


FIG. 1.—Diagram showing arrangement of pedicelled and sessile spikelets in A, undifferentiated four-rowed branch; B, eight-rowed ear, the result of the fasciation of two undifferentiated branches; C, eight-rowed ear the result of twisting a single undifferentiated branch; D, 16-rowed ear, the result of fasciation; E, 16-rowed ear, the result of a further twisting of "C."

Turning now to the pistillate inflorescences a striking contrast appears. Both spikelets are sessile in Zea, and it is usually impossible to determine which of the pair is the homologue of the pedicelled spikelet. In the pistillate inflorescence of Euchlaena, the spikelets are borne singly instead of being paired. It is the pedicelled spikelet that is suppressed, as is clearly shown in the hybrid plants where all stages of suppression can be observed (Pl. 17, A). Furthermore, in pure Euchlaena staminate branches frequently have pistillate spikelets at the base. In such specimens at the place where the transition occurs, rudiments of a pedicelled staminate spikelet can often be seen by the side of a sessile spikelet bearing a well-developed seed.

TWO-RANKED AND MANY-RANKED ALICOLES

The manner by which the number of rows has been increased in the pistillate inflorescence of maize has been the subject of much controversy. Two ways of developing additional rows have been suggested—by fasciation of long lateral branches of a compound inflorescence like the tassel,[1] or by the reduction of branches until each branch is represented by a single pair of spikelets.

The fasciation theory would explain the ear and the central spike of the tassel in the same way, by assuming that a many-rowed spike has resulted from the fusion of simple spikes or branches. In the terminal inflorescence of pure Euchlaena there is no indication of a central spike, all the branches being similar, except that the lower are again subdivided. If two of the upper branches of such an inflorescence were to coalesce, an eight-rowed spike would be formed, and if then the pedicelled spikelets should become sessile and all the spikelets pistillate, an eight-rowed ear would result.

According to the second or reduction hypothesis the development of the ear and the central spike of the staminate inflorescence is supposed to have been accomplished through a shortening of the branches in the upper part of an inflorescence similar to the staminate inflorescence of Euchlaena, the branches being reduced until each was represented by a single pair of spikelets. In apparent conflict with this view is the abrupt transition between the uppermost branch and the lowest spikelets of the central spike, that characterizes all normal varieties of maize. But in the mutation known as *Zea ramosa* the abrupt transition is lost, so that the branches become gradually shorter and pass by imperceptible gradation into simple pairs of spikelets like those of a normal tassel. Thus, *Z. ramosa* may be looked upon as representing an intermediate stage in the formation of a central spike, and as such constitutes the chief support of the reduction theory.

The evidence derived from hybrids of maize and Euchlaena does not support either of these theories. On the contrary, the hybrid plants provide an unbroken series of stages connecting the Euchlaena spike with the maize ear that clearly indicates a third method of increasing the number of rows and forming a central spike or ear. This is by shortening and twisting the rachis of a single spike of Euchlaena, accompanied by an increase in the number of alicoles. The stages in this process will be discussed in more detail later.

SEPARATE AND YOKED ALICOLES

In the pistillate inflorescence of pure Euchlaena the joints of the rachis, each of which bears a single alicole, stand almost directly above one another, resembling a string of triangular beads. One of the most

[1] The earliest published statement of the fasciation theory that has thus far come to light is an anonymous account (Sexual flowers in Indian corn), in Meehan's Monthly, v. 3, p. 105, 1893.

frequent and obvious indications of admixture with maize is a shortening of the rachis. The reduction in length, however, is not uniform but is more pronounced in alternate internodes, with the result that the alicoles become associated and yoked in pairs, the members of which stand nearly opposite to each other.

In the staminate inflorescence of either Euchlaena or the common varieties of maize there is little indication of this yoking of the alicoles. The pairs of spikelets stand on opposite sides of the rachis, but usually they are equally spaced with no indication of yoking, this tendency not even appearing in the pistillate inflorescence of the first generation of the hybrid between maize and Euchlaena. Yoking of the alicoles is, however, a striking characteristic of the second generation and appears in all the stages between the four-rowed spike and a well-formed ear. With the increase in the number of ranks of alicoles this yoking of the alicoles into pairs is obscured, but there are evidences that it still persists even in the fully developed many-rowed ear.

In addition to the sharply contrasted characters discussed above, the pistillate inflorescence of maize differs from that of Euchlaena in having the alicoles much more numerous and more closely crowded.

EUCHLAENA × MAIZE HYBRIDS

Having outlined the nature of the differences between the pistillate inflorescences of Zea and Euchlaena, the pistillate inflorescences of the hybrid plants may now be examined. In the first generation the spikelets are paired, the alicoles separate, and two-ranked. In number of alicoles and degree of crowding they are intermediate between the parents. This mixture of characters derived from both parents creates the general impression that the inflorescence is intermediate.

SECOND AND LATER GENERATIONS

Treating the three contrasted characters of maize and Euchlaena as alternative, there are eight possible combinations: (1) Spikelets single, alicoles separate and two-ranked; (2) spikelets single, alicoles separate and many-ranked; (3) spikelets single, alicoles yoked and two-ranked; (4) spikelets single, alicoles yoked and many-ranked; (5) spikelets paired, alicoles separate and two-ranked; (6) spikelets paired, alicoles separate and many-ranked; (7) spikelets paired, alicoles yoked and two-ranked; and (8) spikelets paired, alicoles yoked and many-ranked. With the exception of No. 6, all of these combinations have been found in second-generation plants and most of them in the descendants of a single cross. To class the individuals into the above eight combinations is, however, a very inadequate expression of the diversity. The dominance shown in the first generation was not followed by any clear-cut segregation in the second. On the contrary, a complete series of intermediates connected the parental forms with respect to each of the three contrasted pairs of characters.

TRANSITION FROM A TWO-ROWED SPIKE TO A MANY-ROWED EAR

The pistillate inflorescence of Euchlaena may be looked upon as a two-rowed ear. In hybrids between maize and Euchlaena the initial step from such a two-rowed ear to one with four rows may be made in two quite different ways. The more common method is for the pedicelled spikelets, which are suppressed in Euchlaena, to reappear. This converts the flat two-rowed spike into a flat four-rowed spike, the condition that obtains in the first generation of the hybrid (Pl. 17, B).

In some instances, however, another method is followed. Alternate internodes of the spike become shortened until the alicoles, each with a single spikelet, are yoked in pairs, the members of which stand opposite or nearly so. The rachis then twists until each pair of alicoles, instead of standing over the one below, stands at right angles with the pair immediately above and below (Pl. 16, D). This results in a square four-row ear. The pairs of alicoles are crossed and fitted into each other in a way that has suggested the name "saddleback" for this type of spike with four rows of alicoles.

In some instances still another step is taken before the spikelets are doubled in the alicole. The rachis is still further shortened and twisted, resulting in a six-rowed ear. Six-rowed ears are sometimes found in which both sessile and pedicelled spikelets are developed. In such cases it appears that the definite relation which ordinarily exists between yoked alicoles has been lost, and starting with the flat four-rowed ear every third alicole has slipped around so that it occupies a plane between the other two, which in turn are slightly displaced (Pl. 17, C).

Returning now to the more common form of a four-rowed ear, it is to be noted that the spike is four-rowed and the pedicels have been shortened, though the distinction between sessile and pedicelled spikelets can still be made out with certainty. The rachis also has been shortened and forced into a series of sharp angles and as a result of such crowding it has now begun to twist (Pl. 17, A).

The next clearly marked stage is the eight-rowed ear. The shortening of the rachis has continued, with increased crowding and twisting of the axil, forcing the alicoles, each bearing a pair of spikelets, to slip past one another into the unoccupied spaces of what were the upper and lower sides of the original horizontal branch. This is again a saddleback type, with the alicoles associated as in the square four-rowed ear described above, though each alicole contains two spikelets instead of one (Pl. 15, F). Intermediate stages between the flat four-rowed ear and the eight-rowed saddleback stage can sometimes be found where the twist is not quite a quarter turn, but all such appear to be unstable. The saddleback, on the contrary, is stable and will sometimes be shown consistently throughout a plant of the second generation (Pl. 18).

A further shortening of the rachis brings about the next stage, which is that of a 10-rowed ear. Intermediate stages are more common during the acquisition of this stage, and when they occur the seeds, as might be expected, are not arranged in regular rows.

With these facts in mind, the spike can be understood as composed of opposite or yoked alicoles, each with a pair of spikelets. These yokes are superposed, and as crowding increases there results a further twisting and the formation of a more complicated spiral. With seeds of a uniform size a compact spiral would result in the formation of longitudinal rows, though these might not run exactly parallel to the axis of the ear, as, indeed, they seldom do even in ears of maize.

MORPHOLOGY OF THE MAIZE EAR

It has been shown that the intermediate forms that appear in hybrids between maize and Euchlaena afford no support for the fasciation theory. Evidence from the ear of pure maize may now be presented.

If a number of four-rowed branches were forced together and their axes united, the conditions found in an ear of maize might result (see fig. 1, B). There is, however, evidence in the ear itself that it is not constructed in this way.

It is not an uncommon occurrence for an ear to drop rows. For example, there may be 12 rows at the base and only 10 rows at the tip. A study of how this transition is made throws light on the morphology of the ear. In the first place, the loss is almost invariably two rows, and both are lost at the same distance from the butt of the ear. There is no region with an odd number of rows. A normal ear is made up of a series of paired rows and this is usually accepted as an adequate explanation of the fact that the number of rows is always even. A pair of rows is looked upon as the fundamental structural unit of the ear, a view in accord with the theory of fasciation. Since two rows are dropped at once, it might be expected that the interrupted rows would be adjacent. This would follow from the suppression of a pair of rows representing the sessile and pedicelled spikelets arising from a single series of alicoles.

There is, however, abundant evidence to show that rows are usually interrupted by the abortion of pedicelled spikelets only. This can be seen in abnormal maize tassels in which the base of the central spike is pistillate, forming in reality a section of an ear. At the place where the transition occurs it can be seen that the sessile spikelets are more persistent and produce larger seeds.[1]

[1] With the idea of determining to what extent differentiation between pedicelled and sessile spikelets persists in the fully developed maize ear, the weight of each of the two seeds from individual alicoles was compared. An ear of flint corn was chosen in which the alicoles were clearly marked and the individual seeds were carefully weighed. There were 135 alicoles with two comparable seeds. The average weight of the individual seeds for all the seeds was 430 mgm. The average difference between the seeds of an alicole was 21.0 mgm. ± 19.5.

It would appear, therefore, that if there was any consistent difference between the weight of the seeds borne in pedicelled and sessile spikelets in this ear, the difference must have been something less than 5 per cent of the weight of the seed.

With the recognition of the fact that the interrupted rows represent pedicelled spikelets instead of the pedicelled and sessile spikelets of a row of alicoles the position of the interrupted rows with respect to one another becomes of importance in studying the formation of the ear.

Following the fasciation theory, if both of the rows of pedicelled spikelets of a single branch aborted leaving the sessile, we should find the two interrupted rows separated by two remaining rows. (This may be illustrated by reference to fig. 1, D. If the two rows of pedicelled spikelets marked *a* were aborted the two missing rows would be separated by two rows.) This is not what occurs. In the examination of many ears in which rows were dropped no instance has been found where the dropped rows were either adjacent or separated by two rows. In cases where the location of the dropped rows can be determined with reasonable certainty the dropped rows are on opposite sides of the ear. Yet they are not exactly opposite, but missing it by just two rows. This is what should occur if the two pedicelled spikelets were dropped simultaneously from a pair of yoked alicoles. It will be recalled that the dorsoventral arrangement of the spikelets in the original four-rowed spike results in bringing the pedicelled spikelets not exactly opposite, but separated by two more rows on one side than on the other. (See fig. 1, E. The pedicelled spikelets of a pair of yoked alicoles are marked *a*. It will be seen that they are separated on one side by six rows and on the other by eight.)

The persistence with which ears of maize maintain an even number of rows is therefore more wonderful than has been supposed, for it can not be fully accounted for by the fact that the spikelets are born in pairs. It must, in addition, be recognized that when a pedicelled spikelet of one alicole is suppressed there is a simultaneous suppression of the pedicelled spikelet in another alicole. The further evidence afforded by Euchlaena hybrids is that the two alicoles are the members of a yoked pair which though standing on opposite sides of the ear, have not lost their identity as a structural and developmental unit.

SUMMARY

Before the pistillate inflorescences of maize and Euchlaena could be compared in detail it was found necessary to recognize as a morphological unit the organs borne by a single metamer of the rachis. This unit, whether staminate or pistillate, whether composed of one or more spikelets, has been called an alicole.

The stages between a Euchlaena spike and a maize ear as they appear in hybrids between the two genera may be summarized as follows:

(1) The suppressed pedicelled spikelet in each alicole reappears. (2) The alicoles become more crowded and their number is increased. (3) The alicoles associate themselves in pairs or yokes. (4) The axis twists, increasing the rows of alicoles.

The order in which these changes occur is by no means fixed, but taken together they comprise all the changes necessary in deriving the maize ear from the Euchlaena spike.

In this series of intermediate stages nothing was observed that affords support for either the fasciation or "reduced branch" theory of ear formation. There is also evidence from the maize ear itself that the association of alicoles into pairs is more fundamental than the linear arrangement.

In all the hybrids between maize and Euchlaena that have been observed there has appeared no suggestion of either pod corn or *Zea ramosa*. Since it can scarcely be doubted that the peculiar characteristics of both of these mutations represent the reappearance of ancestral characters common to the Andropogoneae, it would seem that in crossing maize and Euchlaena, and thus calling forth a series of intermediate forms, we are not returning to the point in the ancestry of maize where it became differentiated from the Andropogoneae.

Furthermore, if the stages shown in the hybrid plants were to be taken as indicating the path of evolution of the ear, it would be necessary to assume that the central spike of the staminate inflorescence or tassel had evolved separately and along different lines. The close homology between the ear and the central spike of the tassel makes such an assumption unreasonable.

In the present article emphasis has been placed on the shortening and twisting of the axis of a single spike as a possible method of deriving a structure like the maize ear from the inflorescence of Euchlaena. This has been done, not because the method is believed to represent the most probable course of evolution, but because the present discussion has been restricted to the evidence afforded by hybrids of maize and Euchlaena, which seems to require such an interpretation.

Facts of other kinds are more easliy interpreted by the theories of fasciation and reduction of branches, but there are also facts that do not seem to accord with any of the theories yet proposed. Until the apparently contradictory evidence can be reconciled, it seems best to keep the several possibilities in mind and await additional evidence before attempting a complete interpretation.

PLATE 16

Intermediate stages between a simple spike of the pistillate inflorescence of Euchlaena and an ear of maize:

A.—Spike of pure Florida teosinte.

B.—Spike with slightly shortened axis.

C.—A still more compact spike with an increased number of seeds. A–C have single spikelets and separate two-ranked alicoles.

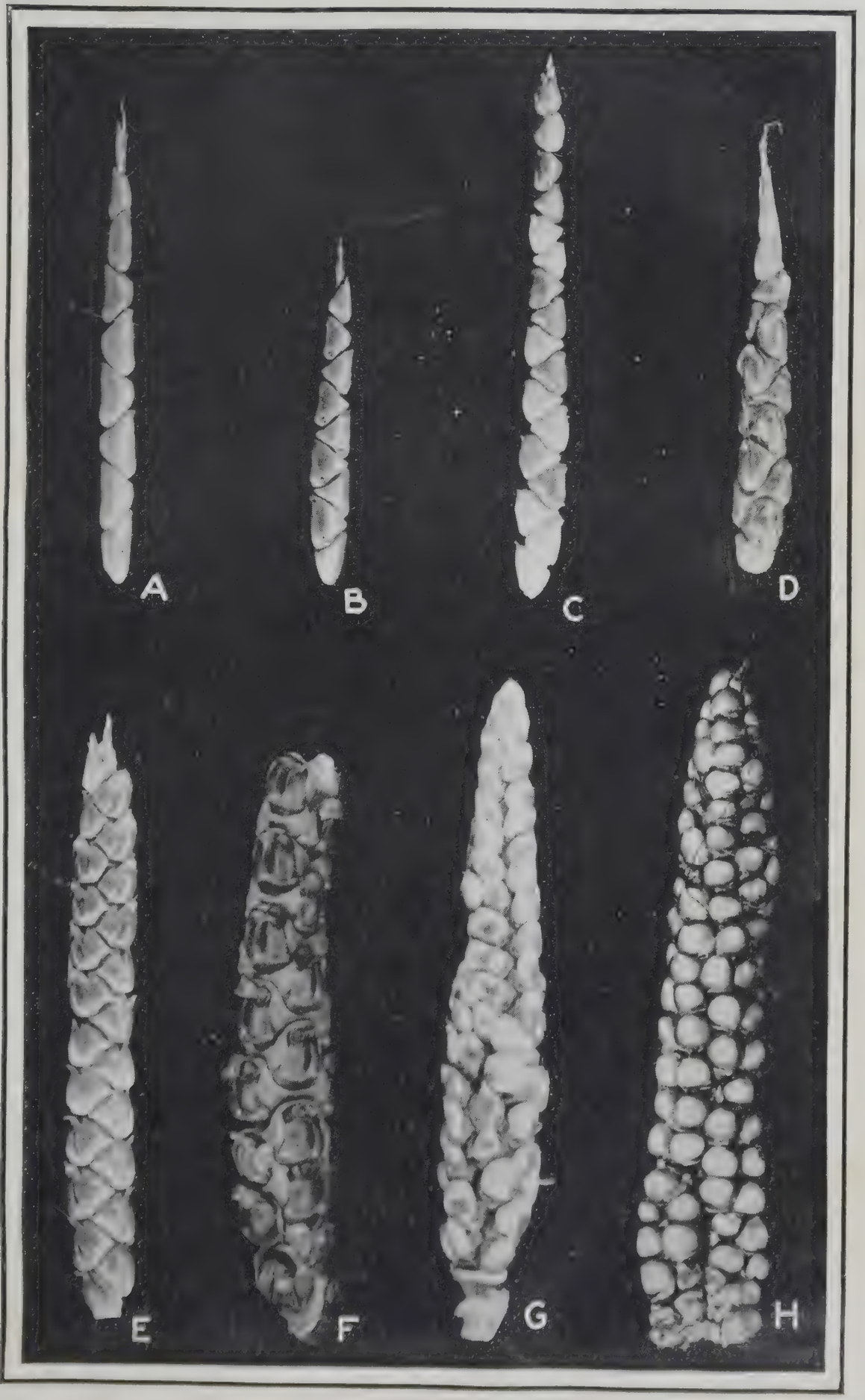
D.—Spike with single spikelets and yoked alicoles, irregularly four-rowed.

E.—Compact spike with two-ranked separate alicoles and single spikelets.

F.—Spike with paired spikelets and four ranks of yoked alicoles.

G.—Transition stage between four-rowed and eight-rowed ear.

H.—Ear of maize with eight rather poorly defined rows of seeds.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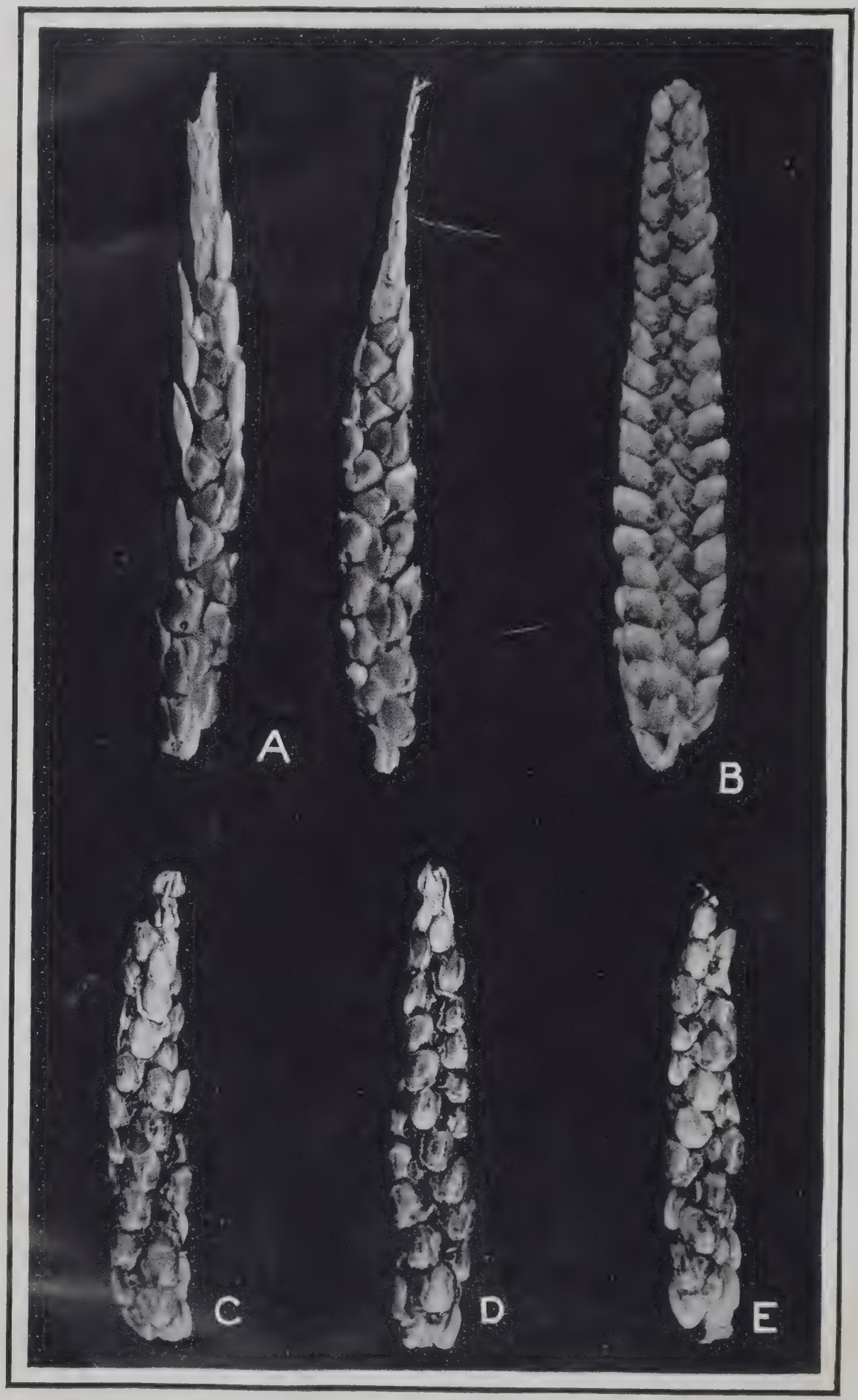
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PLATE 17

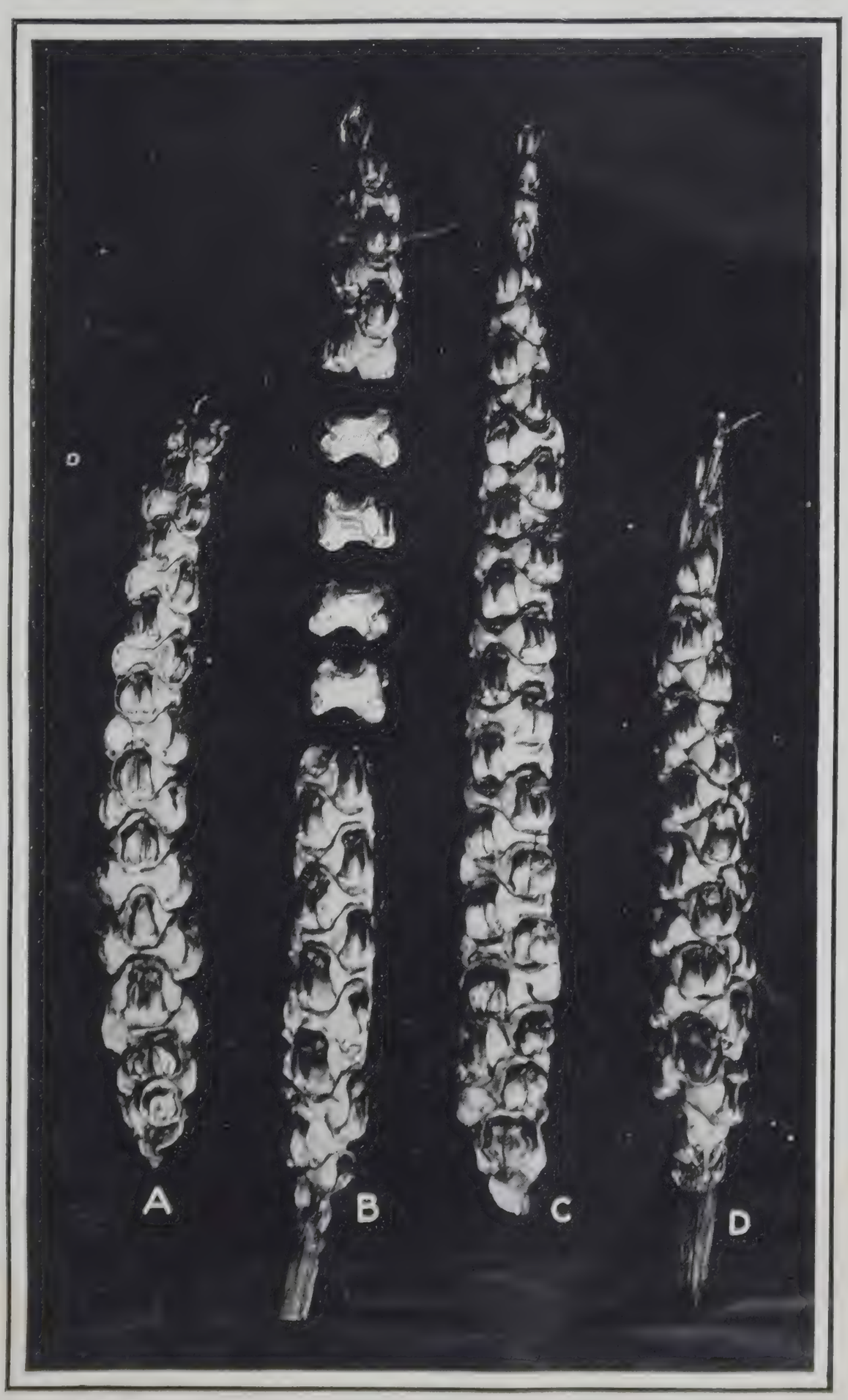
Pistillate inflorescences of hybrid between Euchlaena and maize:
A.—Showing pedicelled staminate spikelets with sessile pistillate spikelets.
B.—Closely compacted inflorescense with two rows of alicoles and four rows of seeds.
C–E.—Spirally twisted inflorescences, with three rows of alicoles.

PLATE 18

Pistillate inflorescences of hybrid between Euchlaena and maize, showing yoked alicoles:

A–C.—The alicoles are in four rows corresponding to an eight-rowed ear.

D.—The alicoles are in five rows, corresponding to a ten-rowed ear.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CARBOHYDRATE METABOLISM IN GREEN SWEET CORN DURING STORAGE AT DIFFERENT TEMPERATURES

By CHARLES O. APPLEMAN, *Plant Physiologist*, and JOHN M. ARTHUR, *Assistant Plant Physiologist, Laboratory of Plant Physiology, Maryland Agricultural Experiment Station*[1]

THE PROBLEM

The present paper deals with the character and kinetics of the processes involved in the rapid depletion of sugar in green sweet corn after it is separated from the stalk and more particularly with the relative rates of these processes at different storage temperatures, accurately controlled.

WORK OF PREVIOUS INVESTIGATORS

In the course of an extensive sweet corn investigation, Straughn[2] clearly shows that the loss of total sugars from green sweet corn is very rapid during the first 24 hours of storage at ordinary summer temperatures. Working with Stowell's Evergreen, he claims that about one-third of the total sugars disappeared during the first 24 hours of storage at a room temperature of about 25° C. A further loss occurred during the next 24 hours, but when the sugars reached 1.80 per cent no further loss was noted. This rate of sugar loss for the first 24 hours of storage at one uncontrolled temperature must be considered as merely an approximation, since analyses of different ears before and after storage were compared. The percentage of sugar in the different ears at the time of picking showed considerable variation. In the same paper it is concluded that there is no material advantage to be gained by storing the corn in a refrigerator. It should be noted, however, that the refrigerator showed a temperature of 23.5° C. during the first 24 hours and thereafter 17° C.

In a later paper by Straughn and Church[3] results are reported showing the change in the sugar content of green corn after a period of 36 hours' storage at room temperature. The data furnish very little additional information on this problem, as the experimental corn was secured upon the open market and the sugar loss in this corn had nearly ceased before

[1] The curves in figures 1 and 2 were drawn by John Paul Jones, of this laboratory.

[2] STRAUGHN, M. N. SWEET CORN INVESTIGATIONS. Md. Agr. Exp. Sta. Bul. 120, p. 37–78. 1907.

[3] STRAUGHN, M. N., and CHURCH, C. G. THE INFLUENCE OF ENVIRONMENT ON THE COMPOSITION OF SWEET CORN, 1905–1908. U. S. Dept. of Agr. Bur. Chem. Bul. 127, 69 p., 11 fig. 1909.

the experiment was begun. However, the data are interesting; they show the usual low sugar content of green corn as it is now purchased on the market. The percentage of sugar in this corn ranged from 1.70 to 1.49.

EXPERIMENTAL METHODS

One of the first problems to solve was a method by which the rate of the carbohydrate changes at different temperatures could be determined without comparing analyses of different ears. The following method was finally adopted: The ears for each experiment were brought to the laboratory within 15 minutes after picking and numbered consecutively. The first set of samples was taken from ears 1 and 2, and all ears were then placed immediately under the experimental conditions. At the end of 24 hours the second set of samples was taken from ears 1 and 2 and the first set from ears 3 and 4. After 48 hours the second set of samples was taken from ears 3 and 4 and the first set from ears 5 and 6. This overlapping method of sampling was continued every 24 hours until the experiment was completed. The change in chemical composition during each consecutive 24-hour period of storage could then be determined by comparing the analytical results of the first and second sets of samples from the same ear.

Stowell's Evergreen corn was stored at seven different temperatures—namely, 0°, 5°, 10°, 15°, 20°, 30°, and 40°C. All the temperatures were controlled within about 1°. The 30° temperature was controlled within 0.1°. The corn was stored with the husks on, and, in the case of the higher temperatures, the ears were placed in large desiccators, with the tubulure on the side left open to allow ventilation. Preliminary experiments showed that, as far as the carbohydrate changes are concerned, active aeration of the small number of ears used in each experiment was not important during the short experimental period of four days.

Under the conditions of the experiments there was very little change in the percentage of water in the corn at any temperature. However, the analytical results from the second set of samples were all calculated to the moisture of the first set in order to avoid false percentages due to loss or gain in water content during storage. In a few cases at the higher temperatures the percentage of water slightly increased on account of the accumulation of respiratory water and possibly water set free by condensation of polysaccharides.

ANALYTICAL METHODS

SAMPLING

Three rows of kernels were removed for each set of samples, care being taken to remove the entire kernel. In order to take the first set of samples, the husks were split lengthwise with a sharp knife and then cut

half way around at the base. After the kernels were removed the husks were brought back to place and held by means of rubber bands. For the second set of samples the husks were removed and three rows of kernels taken from the opposite side of the ear.

The corn was thoroughly ground to a mash in a small unglazed mortar and sampled immediately. On account of the short time required to sample the mash it was found unnecessary to surround the mortar with cracked ice. Each set of samples furnished material for the following determinations: Moisture, total sugars as invert sugar, sucrose, free-reducing substances, and starch. The starch was determined as glucose after hydrolysis with dilute acid.

MOISTURE.—Approximately 5 gm. of the mash were placed between tared watch glasses ground tight and held together by means of a clamp. After weighing, the cover glass was removed and the material covered with 1 cc. of alcohol. The samples were then dried to constant weight in a vacuum at 80° C. During the first drying a stream of warm, dry air was passed through the chamber. The watch glasses were clamped together before each weighing.

SUGARS.—When all things are considered, the alcohol method for the extraction of sugars from plant material in general seems preferable to any other yet devised. Since the procedure by different authors varies considerably, a large number of preliminary experiments were performed to determine the best procedure for the alcoholic extraction of sugars from the particular material at hand—namely, green sweet corn at different stages of maturity. The chief problem was to obtain complete extraction of the sugars and at the same time prevent any inversion of cane sugar as well as diastase action.

These experiments show that there is no appreciable hydrolysis of either sucrose or starch during boiling in 40 or 50 per cent neutral alcohol as long as 60 minutes. However, complete extraction was obtained by a much shorter period of boiling, and consequently the loss of alcohol during extraction is very much reduced.

The procedure finally adopted was as follows: Samples of 16 gm. each were weighed out into counterpoised 200 cc. Kohlrausch sugar flasks. A small amount of calcium carbonate was added to neutralize any acids liberated in the mash. It was later found that in the case of sweet corn this is not as important as in the case of many other plant tissues. The samples were covered immediately with 75 cc. of hot 95 per cent alcohol, the alcohol being previously measured into small boiling flasks and brought to boil on an electric hot plate. After the mixture began to boil on the steam bath, 50 cc. of hot water were added. This brought the extraction alcohol down to about 50 per cent. The water in the sample was taken into consideration in making this calculation. The foregoing method precluded any possible enzyme action in the weak alcohol while heating up to the boiling point. Small funnels were placed

in the necks of the flasks to condense the alcohol and the mixture was allowed to boil 30 minutes. While still hot, the flasks were made up to the mark with 95 per cent alcohol and allowed to stand over night. They were then shaken, again made up to the mark, tightly stoppered, and stored. The final strength of the alcohol in which the samples were stored was about 64 per cent.

When a large number of samples are taken during a comparatively short time, as was the case in this work, it becomes necessary to store most of the samples for some time. Since the storage problem is an important one, a number of experiments were conducted to determine the best treatment of the samples to prevent any carbohydrate changes during long periods of storage. The final method, previously described, was found to preserve the samples for at least 145 days without any appreciable carbohydrate changes. After boiling, the samples may be safely stored in 50 per cent alcohol. Cold treatment of the samples with 52 per cent alcohol inhibited invertase action, but there was considerable starch hydrolysis after a long period of storage. If the number of volumetric flasks is limited, a measured quantity of the filtered extract can be stored. In this work 150 cc. were frequently stored.

The method employed for the determinations of the sugars in the solutions was essentially the same as the one described by Bryan, Given, and Straughn.[1]

STARCH.—Ten gm. of the mash were weighed into counterpoised 200 cc. Erlenmeyer flasks and immediately covered with 50 cc. of 95 per cent alcohol. About 0.05 gm. of calcium carbonate was added and after thorough shaking the flasks were tightly stoppered and stored. The strength of the cold alcohol in the mixture was approximately 80 per cent. This was found sufficient to preserve the samples for several weeks without any appreciable change in the carbohydrates present. The method of weighing out the samples in small flasks, counterpoised on torsion balances sensitive to one-fifteenth gm. was found to give just as good duplicates as weighing the samples to the third place in weighing bottles. By the former method, the samples could be placed in alcohol in a very much shorter time.

The determinations were made according to the following procedure: Decant the preserving alcohol on to a 9 cm. No. 1 Whatman filter paper; add 75 cc. of 50 per cent alcohol and extract 24 hours at room temperature, shaking noon and evening; decant completely the 50 per cent alcohol; add 50 cc. more of the 50 per cent alcohol and allow to stand two hours, shaking three times; decant the alcohol and when all has run through the filter transfer the mash to the filter; apply suction and drain; add 50 cc. of 50 per cent alcohol to the flask to wash down

[1] BRYAN, A. Hugh, GIVEN, A., and STRAUGHN, M. N. EXTRACTION OF GRAINS AND CATTLE FOODS FOR THE DETERMINATIONS OF SUGARS; A COMPARISON OF THE ALCOHOL AND THE SODIUM CARBONATE DIGESTIONS. U. S. Dept. Agr. Bur. Chem. Circ. 71, 14 p. 1911.

the sides and transfer to the filter. With small portions of 50 per cent alcohol, transfer to the filter any material still remaining in the flask; after the alcohol has drained out of the filter, fill up once more with 50 per cent alcohol and drain. All sugars and any other reducing materials are now removed from the residue on the filter. The filter is filled twice with 95 per cent alcohol and the residue allowed to dry on the filter. The filter paper may be folded over the sample and placed in small stoppered vials for another period of storage if necessary.

The filter paper containing the sample was placed in a Kjeldahl flask and covered with 200 cc. of water; sufficient hydrochloric acid was added to give a final strength of acid in the mixture of 2.5 per cent. Hydrolysis was effected by boiling under a reflux condenser for three hours.

A number of the filter papers used for the filtration were hydrolyzed in the same strength of acid and for the same length of time as the samples. Although the papers were claimed by the manufacturers to be starch free, they were found to give a small amount of reducing material after hydrolysis. However, the amount of this material was consistent in all the boxes and in different parts of the box, so it was very easy to make the necessary correction for the filter paper in the final results. The starch was determined as glucose, but of course it includes any other polysaccharides which furnished reducing substances during the acid hydrolysis.

EXPERIMENTAL DATA

The work had not progressed far until it was evident that if the moisture in the corn at the time of picking had fallen below a certain percentage it became a factor in controlling the rate of sugar loss. In order to eliminate this variable factor, so that attention could be focused upon the temperature relation, the experimental ears were carefully selected to represent a fairly definite stage of maturity—namely, the typical milk or best eatable stage. Ears falling below 80 per cent water were excluded from the final calculations.

The work of the first year was repeated on another crop the succeeding year. The results of the two years' work are averaged in Table I. In the experiments of the first year, the carbohydrate changes for each consecutive 24-hour period were not determined in duplicate ears as described for the experiments of the second year. Each percentage in the table, therefore, is the mean of three ears, except in a very few cases where the results of one ear were excluded on account of the moisture content's falling below the arbitrary standard. The results of the experiments at 5° and 15° C. are not given, as they add nothing to the general conclusions. The average percentage of sugars in the corn at the beginning and end of each storage period is indicated by (*a*) and (*b*), respectively.

TABLE I.—*Loss of sugar from green sweet corn during consecutive 24-hour periods of storage at different temperatures*

ALL SUGARS

Number of hours in storage.	Ear lot.	Storage temperatures. 0° C. Total.[1]	0° C. Loss.[1]	10° C. Total.[1]	10° C. Loss.[1]	20° C. Total.[1]	20° C. Loss.[1]	30° C. Total.[1]	30° C. Loss.[1]	40° C. Total.[1]	40° C. Loss.[1]
0	1*a*	5.91		5.83		6.17		5.34		6.72	
24	1*b*	5.43	0.48	4.83	1.00	4.59	1.58	2.65	2.69	3.64	3.08
24	2*a*	6.70		3.95		3.68		3.11		2.30	
48	2*b*	5.96	.74	3.43	.52	2.69	.99	2.68	.43	1.69	.61
48	3*a*	6.63		4.61		3.07		2.10		2.00	
72	3*b*	6.36	.27	3.92	.69	2.52	.55	2.03	.07	1.90	.10
72	4*a*	6.10		3.54		2.24		1.59			
96	4*b*	5.75	.35	2.93	.61	1.97	.27	1.49	.10		

SUCROSE

0	1*a*	3.87		3.77		3.68		3.68		4.50	
24	1*b*	3.73	0.14	3.00	0.77	2.54	1.14	1.50	2.18	2.18	2.32
24	2*a*	4.06		2.53		1.84		1.52		1.18	
48	2*b*	3.77	.29	1.99	.54	1.17	.67	1.24	.28	.76	.32
48	3*a*	4.49		2.74		1.38		1.02		1.05	
72	3*b*	4.25	.24	2.30	.44	1.12	.26	.97	.05	.91	.14
72	4*a*	3.84		1.87		1.11		.71			
96	4*b*	3.56	.28	1.41	.46	.85	.26	.67	.04		

FREE-REDUCING SUBSTANCES

0	1*a*	1.84		1.85		2.07		1.65		1.98	
24	1*b*	1.70	0.14	1.68	0.17	1.77	0.30	1.16	0.49	1.32	0.66
24	2*a*	1.66		1.29		1.68		1.56		1.06	
48	2*b*	1.55	.11	1.28	.01	1.41	.27	1.42	.14	.80	.26
48	3*a*	1.91		1.73		1.55		1.07		.90	
72	3*b*	1.89	.02	1.50	.23	1.28	.27	1.04	.03	.93	.00
72	4*a*	2.05		1.57		1.13		.88			
96	4*b*	2.00	.05	1.45	.12	1.04	.09	.81	.07		

[1] Total quantities of all sugars before and after storage and losses during storage are expressed in percentages.

The data in Table I show that the loss of sugar from corn during storage is not uniform, but becomes slower and slower as a final equilibrium is approached. The relative rates of processes of this kind at different temperatures can be determined accurately only by comparing

the times required to perform equal amounts of work at all temperatures, and not by comparing the amounts of work performed in equal times. In other words, we must compare the times required at the different temperatures to bring the process to the same stage. We are thus comparing stages where the ratio between the reacting material and the products is the same. Osterhout [1] has recently emphasized this point in a "Note on measuring the relative rates of life processes."

In order to make it possible to determine, on this basis, the relative rates of sugar loss at the different temperatures, the experimental results in Table I can be easily interpolated by a simple graphic method, to be described later, if they can be expressed in curves all starting from the same point.

This could be decided only atter a careful consideration of all the factors involved. If mass action alone were responsible for the gradual decline in the rate of sugar loss, then, at a given temperature, the average rate of change in any unit of time would be proportional to the sugar concentration. For a range of original sugar from about 4.5 to 7 per cent and of water from 78 to 80 per cent this was found to be the case for the first 48 hours of storage even at 30° C. (Table II).

TABLE II.—*Proportion of sugar lost during first 48 hours of storage at 30° C.*

Ear No.	Reducing sugar before storage.	Loss during storage.		Total sugar before storage.	Loss during storage.		Sucrose before storage.	Loss during storage.	
		Actual.	Proportional.		Actual.	Proportional.		Actual.	Proportional.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	1.70	0.68	37	6.16	3.59	58	4.24	2.77	65
2	2.40	1.02	42	7.20	3.87	54	4.80	2.95	61
3	2.60	1.05	40	6.74	3.88	58	4.14	2.89	69
4	.96	.22	43	4.47	2.59	58	3.33	2.33	69
5	1.66	.82	49	5.91	3.41	58	4.25	2.68	63
6	3.00	1.31	44	6.55	3.69	57	3.55	2.43	68

The sugar loss ceases when an appreciable amount of sugar is still present. Therefore, the speed of the counter process, that is, the formation of sugar, becomes a factor to be reckoned with when the processes have nearly reached an equilibrium. If at the beginning of storage the percentage of sugar in ear 1 is considerably greater than in ear 2, the latter would reach the equilibrium position sooner than ear 1. At the end of 72 hours of storage at 30° C. ear 1 might still have 2 per cent sugar while in ear 2 the sugar content might be only 1 per cent. The sugar loss in ear 2 being nearer the equilibrium point, the speed of the counter process would be greater in this ear than in ear 1. Therefore, during the next 24 hours the proportionality between the sugar lost and the sugar

[1] OSTERHOUT, W. J. V. NOTE ON MEASURING THE RELATIVE RATES OF LIFE PROCESSES. *In* Science, n. s., v. 48, no. 1233, p. 172–174, 3 fig. 1918.

present would not be the same in the two ears. This was proved experimentally.

In considering the rate of the counter reaction in connection with the problem at hand—namely, the possibility of expressing the experimental results in curves all starting from the same point—it must be borne in mind that it becomes appreciable only near the point of equilibrium, and even then it would affect the proportionality between the sugar present and the sugar lost in different ears at the same temperature only when the percentage of sugar in the ears at the beginning of storage varied considerably.

A decrease in the quantity of active enzymes present would produce a steady fall in the values of the velocity constants; this would cause a decreasing rate of actual sugar loss. There is no evidence that this occurs up to 30° C.

In view of the foregoing facts, together with the fact that the ears selected for the final calculations were all in practically the same stage of maturity and therefore contained nearly the same percentage of original sugar, the following procedure in preparing the data for construction of curves all starting from the same point seemed justified. The sugar lost during each 24-hour period of storage was calculated as proportions of the sugar present in the ears at the beginning of each period. The percentages of sugar found in the ears analyzed at the beginning of each experiment were then averaged. Ten ears with not less than 80 per cent water were included in the final average.

Taking this mean as the starting point for all temperatures and applying the proportions of sugar lost during each succeeding 24-hour period, calculated from the experimental data, a new set of proportions was obtained, based upon the same original sugar content in all cases. A single concrete case may serve to clarify the foregoing procedure. The total sugar in all the ears analyzed at the beginning of each experiment averaged 5.766 per cent. During the first 24 hours of storage at 30° C. the average loss of total sugar in three ears was 50.28 per cent of the initial sugar present; that is, the total sugar in the corn was 50.28 per cent less than at the beginning of storage. Applying this proportion to an initial sugar content of 5.766 per cent, we obtain, after the first 24-hour period of storage at 30°, a total sugar content of 2.867 per cent. Making use of all the experimental proportions in the same manner, the percentage of total sugar present at the end of each 24-hour period of storage was calculated, assuming that the sugar content at the beginning of storage was 5.766 per cent. Each calculated percentage was then substracted from 5.766, the initial sugar present. The sugar loss, expressed as percentages of the initial sugar, could then be calculated for the following storage periods: 24, 48, 72, and 96 hours. The same procedure was followed for all the sugars at all the temperatures, with the results shown in Table III.

TABLE III.—*Sugar loss from green sweet corn during different periods of storage at different temperatures, expressed as percentages of the same initial sugar at all temperatures*

TOTAL SUGARS

Number of hours in storage.	Storage temperature.				
	0° C.	10° C.	20° C.	30° C.	40° C.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
24	8. 12	16. 98	25. 61	50. 28	45. 79
48	14. 51	27. 95	45. 73	57. 09	60. 15
72	18. 03	38. 71	55. 50	59. 00	62. 16
96	22. 00	49. 22	62. 10	61. 84	.....

SUCROSE

24	3. 51	20. 78	31. 05	59. 42	51. 03
48	10. 39	37. 49	56. 12	66. 76	64. 68
72	15. 08	47. 46	64. 22	68. 55	69. 24
96	21. 25	60. 54	70. 16	70. 19	.....

FREE-REDUCING SUBSTANCES AS INVERT SUGAR

24	7. 58	9. 26	14. 72	29. 96	33. 48
48	13. 61	10. 52	28. 84	36. 19	49. 76
72	14. 62	16. 71	40. 79	39. 74	49. 76
96	16. 97	23. 33	45. 65	43. 19	.....

The data in Table III, showing the rate of actual loss for total sugars and sucrose, were plotted as curves (fig. 1 and 2).

The curve for 0° C. shows a more rapid sugar loss than is typical for this temperature. In the first place, it required some time for the corn to cool down to this temperature. At the end of each 24-hour period a pair of ears were removed from the cold chamber in order to take the first set of samples. Although the sampling period was short, the temperature of the corn would soon rise a few degrees above 0°. The loss of sugar at the sampling temperature is accumulative in the curve.

The inversion of sucrose appears to be the controlling process in the sugar loss, as the curves for the decrease of sucrose are very similar to those for the loss of total sugar.

TEMPERATURE COEFFICIENT.—Since the curves in figures 1 and 2 all start from the same point, by means of a simple graphic method the relative rates of sugar loss at the different temperatures can now be determined by comparing the times at different temperatures required to do the same amount of work. As an illustration we will choose a stage in the depletion of sugar when 40 per cent of the total sugar is lost; in other words, at this point the sugar in the corn is 40 per cent less than at the beginning of storage. A horizontal line is drawn from

this point through all of the curves. Vertical lines are now dropped from the points of intersection to the base line. The times in hours required at the different temperatures to bring the sugar loss to this point are read off on the base line (see fig. 1). The procedure was repeated for all the percentages given on the ordinate.

The relative rates of sugar loss at the different temperatures are expressed in Table IV as the reciprocals of the times in hours required to bring the process to five different stages. The temperature coefficients were obtained from these reciprocals. The results at 40° C. were not

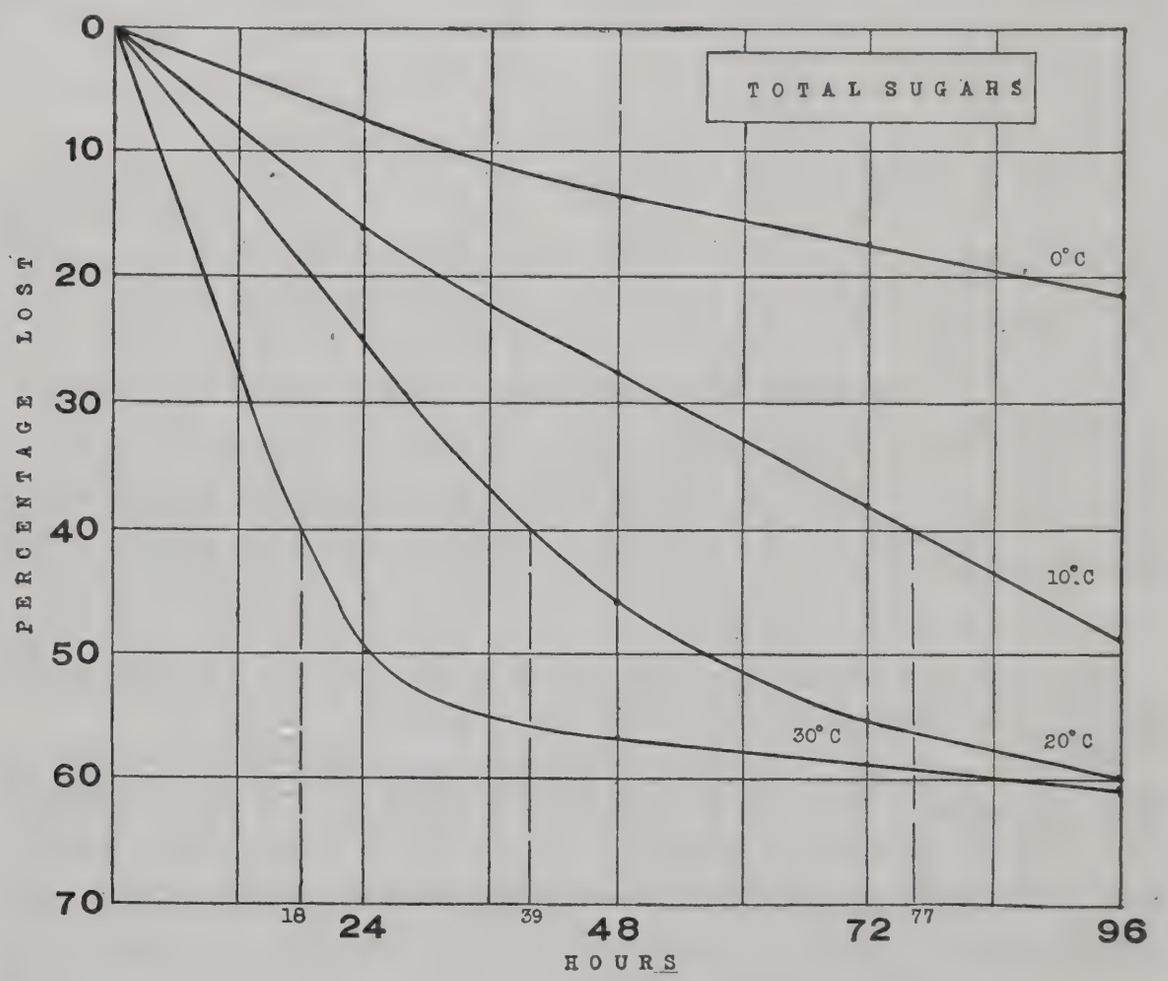

FIG. 1.—Depletion of total sugars in green sweet corn during consecutive 24-hour periods of storage at different temperatures. The ordinates are given by the numbers on the left of the figure and represent the loss of sugar expressed as percentages of the initial sugar, which was 5.91 per cent, wet weight.

included in the foregoing calculation as there was evidently destruction of the enzymes or other alteration in the system by the high temperature.

Some of the curves for the sugar loss, expecially those for sucrose, approach true logarithmic curves; and satisfactory constants were obtained for most of the storage period by applying the simple uni-molecular equation. During the latter part of the period there was a falling off in the velocity constants, due no doubt to the counter reaction. The simple uni-molecular equation assumes that the reaction proceeds to completion or so near completion that the speed of the counter may be ignored. However, as Osterhout has shown in the paper previously

cited, it is not necessary to determine the true velocity constants of a process under different conditions if only the relative rates are desired. This may be accomplished in the manner indicated in Table IV by comparing the reciprocals of the times required to do the same amount of work.

In general, it may be stated that up to 30° C. the rate of sugar loss in green corn is doubled for every increase of 10°. This applies to both total sugars and sucrose. It should be noted, however, that between 0° and 10° the temperature coefficient for sucrose is considerably greater than 2.

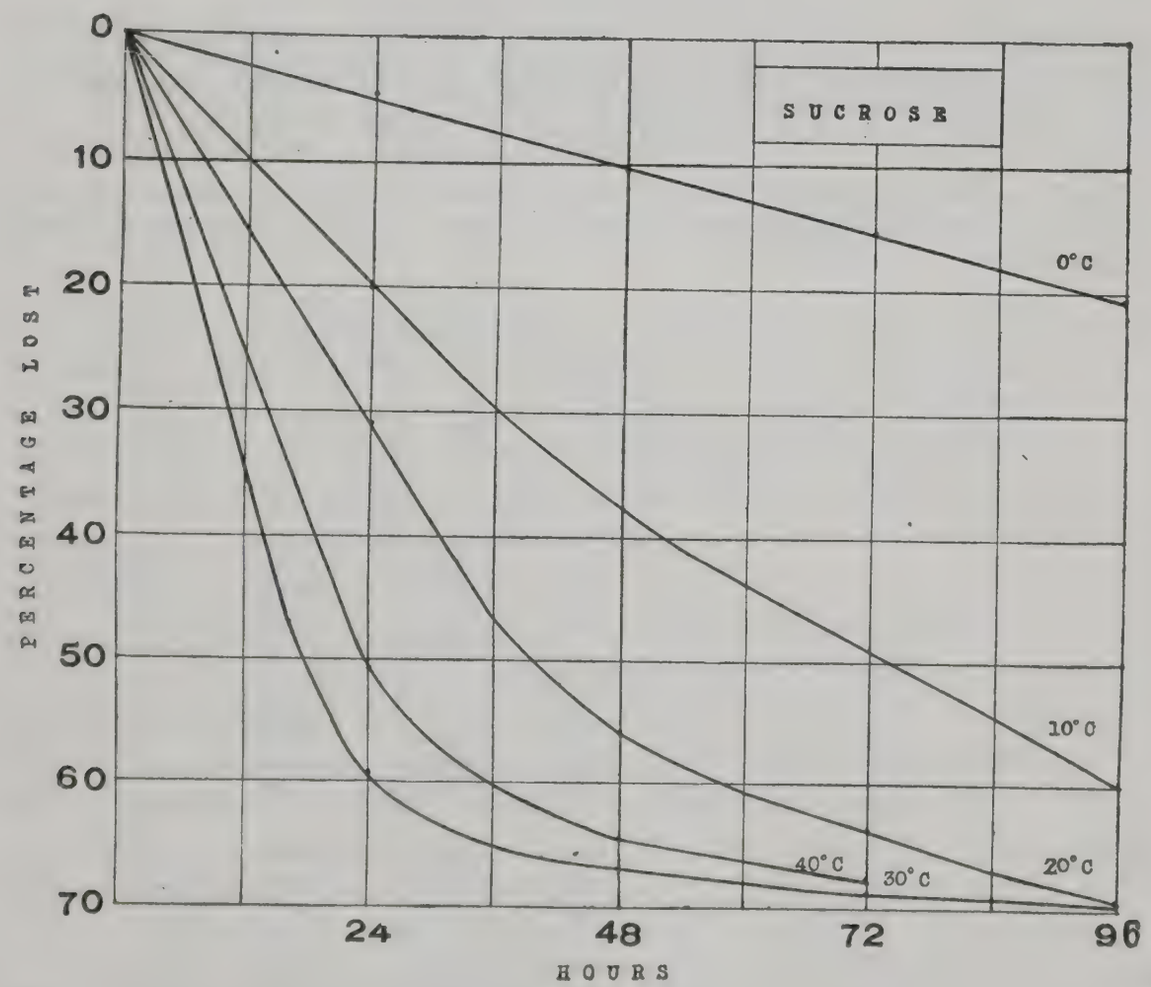

FIG. 2.—Depletion of sucrose in green sweet corn during consecutive 24-hour periods of storage, expressed as percentages of the initial sucrose in the corn, which was 3.87 per cent, wet weight.

RESPIRATION.—In a former paper the writer [1] has shown that respiration in green sweet corn after it is first pulled from the stalk is comparatively high. During the first 24 hours of storage at 30° C. the corn with the husks removed respired at an average rate of 50 mgm. of carbon dioxid per kgm. per hour. This rate became slower and slower until it reached, in eight days, a constant rate of about 18 mgm. of carbon dioxid per kgm. per hour. Respiration of course consumes sugar and therefore accounts for some of the depletion of sugar in sweet corn

[1] APPLEMAN, Charles O. RESPIRATION AND CATALASE ACTIVITY IN SWEET CORN. *In* Amer. Jour. Bot., v. 5, p. 207–209, 1918.

during storage. During each consecutive 24-hour period of storage the percentage of sugar in the corn, however, is only slightly altered by respiration, as shown by the following illustration. Straughn, in the paper previously cited, averaged the weight of kernels and cobs from 18 ears and found that the kernels averaged approximately 50 per cent of the total weight. If we assume that all of the carbon dioxid came from the kernels, then 500 gm. of kernels would produce 1,200 mgm. of carbon dioxid during the first 24 hours' storage at 30°. From the formula

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

1,200 mgm. of carbon dioxid would correspond to the consumption of 818.61 mgm. of sugar. The consumption of this amount of sugar by respiration would free in the system 491.343 mgm. of water.

TABLE IV.—*Reciprocals of the times, in hours, required at different temperatures to bring the sugar depletion in sweet corn to five different stages. Also the temperature coefficients obtained from these reciprocals*

Percentage of initial sugar lost.	Storage temperature.	Reciprocals of time periods.		Temperature coefficients.	
		Total sugars.	Sucrose.	Total sugars.	Sucrose.
	°C.				
10	0	0.0303	0.0213	.....	.....
	10	.0666	.0833	2.2	3.91
	20	.1041	.1282	1.56	1.53
	30	.2083	.2777	2.00	2.16
20	0	.0122	.0108	.....	.....
	10	.0320	.0416	2.62	3.85
	20	.0520	.0666	1.62	1.60
	30	.1111	.1388	2.13	2.08
30	10	.0184	.0268	.....	.....
	20	.0354	.0427	1.92	1.59
	30	.0724	.0925	2.04	2.16
40	10	.0131	.0191	.....	.....
	20	.0252	.0326	1.92	1.71
	30	.0555	.0694	2.30	2.12
50	10	.0101	.0136	.....	.....
	20	.0173	.0256	1.71	1.88
	30	.0406	.0555	2.34	2.17
60	10	.....	.0104	.....	.....
	20	.....	.0171	.....	1.64
	30	.....	.0416	.....	2.43
Average temperature coefficient		.....	.....	2.04	2.14

For the sake of simplicity, we will confine the system to 100 gm. of corn and suppose that at the beginning of storage it contained 5 gm. or 5 per cent sugar and 80 gm. or 80 per cent water, a fair average for the

corn used in this work. According to the foregoing rate of respiration this system would lose 163.72 mgm. of sugar during the first period of storage of 24 hours. At the same time 98.269 mgm. of water would be freed in the system. Our system would now contain 80.0983 gm. of water and 4.8363 gm. of sugar. By correcting for the slight loss of dry matter, the system would contain 80.1507 per cent water and 4.8395 per cent sugar. These percentages would be those found by actual analysis of the 100 gm. of corn after 24 hours' storage, assuming that no other changes occurred besides respiration.

If we now calculate the percentage of sugar on the basis of the original water in the system, as was done in all cases in this work, the percentage of sugar would be 4.8726, showing a loss by respiration of 0.1274 per cent.

It should be noted that the rate of respiration chosen for this illustration was the rate for the highest period at 30° C. It was also assumed that all of the carbon dioxid came from the kernels. During the immature stages of the corn it is very probable that some of the sugar in the cob is consumed by respiration.

During the later periods at the high temperatures and for all periods at the low temperatures, the change in the percentage of sugar by respiration during the short periods of 24 hours would be practically within the experimental error for the sugar and moisture determinations.

One ton of husked green sweet corn, during the first 24 hours of storage at 30° C. would lose approximately 3.2 pounds of sugar on account of respiration.

Under certain conditions, however, respiration may become an important factor in accelerating the depletion of sugar from green sweet corn. One of the products of respiration is heat. This heat of respiration will raise the temperature on the inside of large piles of green corn to a very marked degree. The increased temperature accelerates not only the respiratory process itself but also the other processes responsible for most of the sugar loss. Aeration of green corn is therefore important in order to dissipate the heat of respiration. In other words, green corn should not be allowed to remain in large piles for even a short time, especially during midsummer temperature.

STARCH FORMATION.—If the sugar is all converted into starch or other polysaccharides, hydrolyzed by dilute acids, then the sum of the total sugars and the polysaccharides as glucose should be the same before and after storage. During the first period there is a slight deficit after storage, especially in the more immature ears. A part of this deficit is due to the high respiration of this period; but some of it is probably accounted for, in the immature ears when the sugar is high, by the formation of cellulose. During the later periods of storage many of the ears, depending largely upon the stage of maturity, show a slight increase in the sum of the total sugars and polysaccharides. This is true especially at the higher temperatures and is probably accounted for by the

sugar of the cob being drawn into the grain for starch formation as the sugar in the grain is depleted. Analyses of cobs from immature ears gave a total sugar content of about 7 per cent. The sugar in the cob decreased slightly during storage, but there was no starch formation in the cob.

After noting these exceptions, which alter the balance only slightly, it may be stated in general that most of the sugar loss in green sweet corn is balanced by the gain in polysaccharides, chiefly starch (Table V).

TABLE V.—*Depletion of sugar in green sweet corn balanced chiefly by formation of polysaccharides hydrolyzed by dilute acid*

0° C.

Ear No.	Total sugars plus polysaccharides as glucose.							
	First period.		Second period.		Third period.		Fourth period.	
	0 hours.	24 hours.	24 hours.	48 hours.	48 hours.	72 hours.	72 hours.	96 hours.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	11.01	11.04	12.40	12.39	11.07	11.29	13.57	13.56
2	10.72	10.67	14.84	15.35	.....	.....	13.41	12.68
Average.	10.86	10.86	13.62	13.87	11.07	11.29	13.49	13.12

10° C.

1	14.56	14.53	15.26	15.89	15.86	15.35	14.29	15.05
2	11.79	11.37	14.01	13.50	11.93	11.58	10.81	10.49
Average.	13.18	12.95	14.64	14.70	13.89	13.47	12.55	12.77

20° C.

1	11.50	11.09	15.19	15.13	11.28	10.56	10.93	10.46
2	10.82	9.97	13.64	13.58	10.21	10.24	13.34	13.76
3	.....	.....	12.13	11.20	12.67	12.20	.....	.....
Average.	11.16	10.53	13.65	13.30	11.35	11.00	12.14	12.11

30° C.

1	14.75	14.10	13.39	13.55	13.30	13.32	12.48	12.66
2	12.20	10.53	16.77	17.24	12.16	12.66	12.17	12.61
3	.....	.....	13.13	12.01	10.80	12.36	11.43	12.17
Average.	13.48	12.31	14.43	14.26	12.08	12.78	12.02	12.48

SUMMARY

The data recorded in this paper apply to Stowell's Evergreen corn, picked in the typical milk or best eatable stage and having a water content of approximately 80 per cent.

A method was devised by which the rate of sugar loss from green sweet corn could be determined for consecutive 24-hour periods of storage by comparing analyses of corn from the same ear.

The depletion of sugar in green sweet corn after it is separated from the stalk does not proceed at a uniform rate but becomes slower and slower until finally the loss of sugar ceases when the initial total sugar has decreased about 62 per cent and the sucrose about 70 per cent. Calculated on the basis of original moisture, the corn contained, when the depletion of sugar ceased, approximately 1.5 per cent total sugar as invert sugar, 0.7 per cent sucrose, and 0.8 per cent free-reducing substances. The actual percentage of sugars would depend upon the amount of water in the corn after storage. Under the experimental conditions there was very little change in the percentage of water in the corn employed in this work.

Reversibility of the chief processes involved in the sugar depletion, resulting in an equilibrium between the rate of sugar loss and the rate of sugar formation, would account for the cessation of actual sugar loss.

During the early periods of storage, the falling off in the rate of actual sugar loss is due to mass action. When the equilibrium is nearly reached the counter reaction, that is the formation of sugar, also tends to slow up the rate of sugar loss. Any destruction or decrease in the quantity of enzymes present would produce a falling off in the value of the velocity constant, with a consequent decrease in the rate of actual sugar loss. There is no evidence that this occurs up to 30° C. At 40° there is actual destruction of the enzymes or other alteration on the system. The rate of actual sugar loss must not be confused with the velocity constant.

Raising the temperature simply hastens the attainment of the equilibrium positions, which seem to be about the same for all temperatures. At 30° C., 50 per cent or most of the total sugar loss occurs during the first 24 hours of storage. At 20°, 25 per cent, and at 10°, or good refrigerator temperature, only about 15 per cent is depleted during the same period.

Relative rates at different temperatures, of processes that become slower and slower until an equilibrium is reached, can be accurately determined throughout this entire course only by comparing the times required to bring the process to the same stage at all temperatures. In order to make this comparison possible the experimental results were interpolated by a simple graphic method. The temperature coefficient was then obtained by comparing the reciprocals of the times

required to do the same amount of work at the different temperatures. In this manner the temperature coefficients were determined for six different stages. Up to 30° C. an average coefficient of 2.03 was obtained for the loss of total sugars and 2.14 for sucrose. From 0° to 10° it was greater than 2 in the case of sucrose.

In general, it may be stated that the rate of sugar loss, until it reaches 50 per cent of the initial total sugar and 60 per cent of the sucrose, is doubled for every increase of 10° up to 30° C.

Respiration in green corn is comparatively high when the corn is first picked but falls off rapidly with storage. This process, however, accounts for only a small part of the actual decrease in the percentage of sugar in the corn during the consecutive 24-hour periods of storage even at 30° C. One ton of husked green sweet corn during the first 24 hours of storage at 30° would lose approximately 3.2 pounds of sugar on account of respiration.

Respiration may become indirectly a more important factor in accelerating the depletion of sugar by raising the temperature on the inside of large piles of green corn.

Most of the decrease in the percentage of sugar in green sweet corn during storage is due to condensation of polysaccharides, chiefly starch.

CERTAIN RELATIONSHIPS BETWEEN THE FLOWERS AND FRUITS OF THE LEMON[1]

By HOWARD S. REED

Professor of Plant Physiology, University of California

The physiological characteristics of the cultivated lemon make it an interesting object for study, since its period of blossoming and fruiting extends through much of the year. On most lemon trees it is possible to find all stages of development between blossoms and mature fruit throughout the year, though in varying amounts. There are distinct cycles in both the vegetative and fruiting activities of this tree whose limits are recognized by those engaged in its cultivation. One of the objects of the present study was to obtain quantitative records of these cycles and especially of the relations between flowers and fruit.

The present study attempts to discuss:

(*a*) The seasonal distribution of the fruit buds;

(*b*) The size and productiveness of the inflorescences;

(*c*) The time required for the growth of fruit and the relation of this time to the season at which the buds appear;

(*d*) The numerical ratio of flower buds to mature fruit.

The material studied consisted of a small group of Lisbon lemon trees located on the Limoneira ranch near Santa Paula, Calif. The trees stand in the midst of a large lemon grove and have received good orchard treatment with respect to cultivation, irrigation, and so forth. No especial attention in these particulars was given to the trees during the time observations were being made. All were free from injurious insects and fungous diseases. Each month for one year approximately 50 fruit twigs bearing fruit buds ready to open were selected and marked with identification tags. The twigs were chosen on seven adjacent trees, six of which were full-bearing trees 22 years old. The seventh was 6 years old, but was very fruitful. As soon as a twig was selected an entry was made on a special blank on which full records could be subsequently kept concerning leaves, buds, fruits, and new twigs. Once a month the twigs were examined and the data recorded on special blanks.

When the first year ended 12 lots of twigs had been selected and marked, and from that time the records on all twigs were continued for another year. Thus the first twigs selected were under observation for two years and the last for one year. A total of 610 twigs was selected and observed, but there was some loss due to the removal of tags by winds, so that the final number was somewhat less than 600. The partial records of twigs

[1] Paper No. 54, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

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whose tags disappeared were discarded. The writer is indebted to the management of the Limoneira Co. for their friendly cooperation in this work, as well as to various members of the staff of the Citrus Experiment Station for their assistance in the tedious work of obtaining and compiling data.

All biological work, especially work done in the field, is accompanied by inevitable error. The present is no exception. In August, 1916, much of the small fruit on these trees was killed during their fumigation with hydrocyanic-acid gas to kill insects. The following November the trees blossomed profusely, perhaps due to the earlier loss of a portion of their crop. In the early months of 1917 some of the small fruit was killed by freezing temperatures, in spite of the fact that oil heaters were used in the grove and all vigilance was exercised to avoid losses. In June, 1917, following a period of very hot weather, much of the more mature fruit fell from the trees. It might be thought advisable to discard a portion of the records which are known to be subject to these errors, but why should one discard errors due to climatic conditions which he recognizes while retaining other possibly greater errors which he does not recognize?

SEASONAL DISTRIBUTION OF FRUIT BUDS

The lemon tree continually produces fruit buds, yet their distribution through the year is not uniform. Information upon their seasonal distribution was obtained from the data for 4,545 "new buds"; that is, buds which appeared on twigs subsequent to the selection of these twigs for the purpose of study. These data were used to avoid the results of conscious or unconscious selection by the person who chose and tagged the original twigs. For example, because large clusters of buds are more conspicuous, a larger percentage of the fruit buds on the tree may have been chosen at one time than at another. The effect of this would have been to give larger records at one season and lower at another. It should be remarked, however, that since we were dealing with what is recognized as "fruit wood" the average number of buds on the twigs selected might be higher than for the average twig of the tree.

The figures given in Table I show the percentage of the new buds which were produced in the different months of the year and are based upon the observations of 4,545 buds during a period of two years.

TABLE I.—*The distribution of lemon buds by months*

[Average for 1916 and 1917]

Month.	Buds (percentage of total).	Month.	Buds (percentage of total).
January	0.31	July	4.81
February	.37	August	2.08
March	29.74	September	1.18
April	36.13	October	1.83
May	4.55	November	13.11
June	3.00	December	2.56

A survey of this table and of figure 1 shows that there are two periods in the year at which fruit buds were principally produced. In round numbers, about 66 per cent of the buds appeared in March and April, about 13 per cent appeared in November, and 20 per cent between April and November. There was, therefore, a very pronounced seasonal distribution of fruit buds on the trees observed.

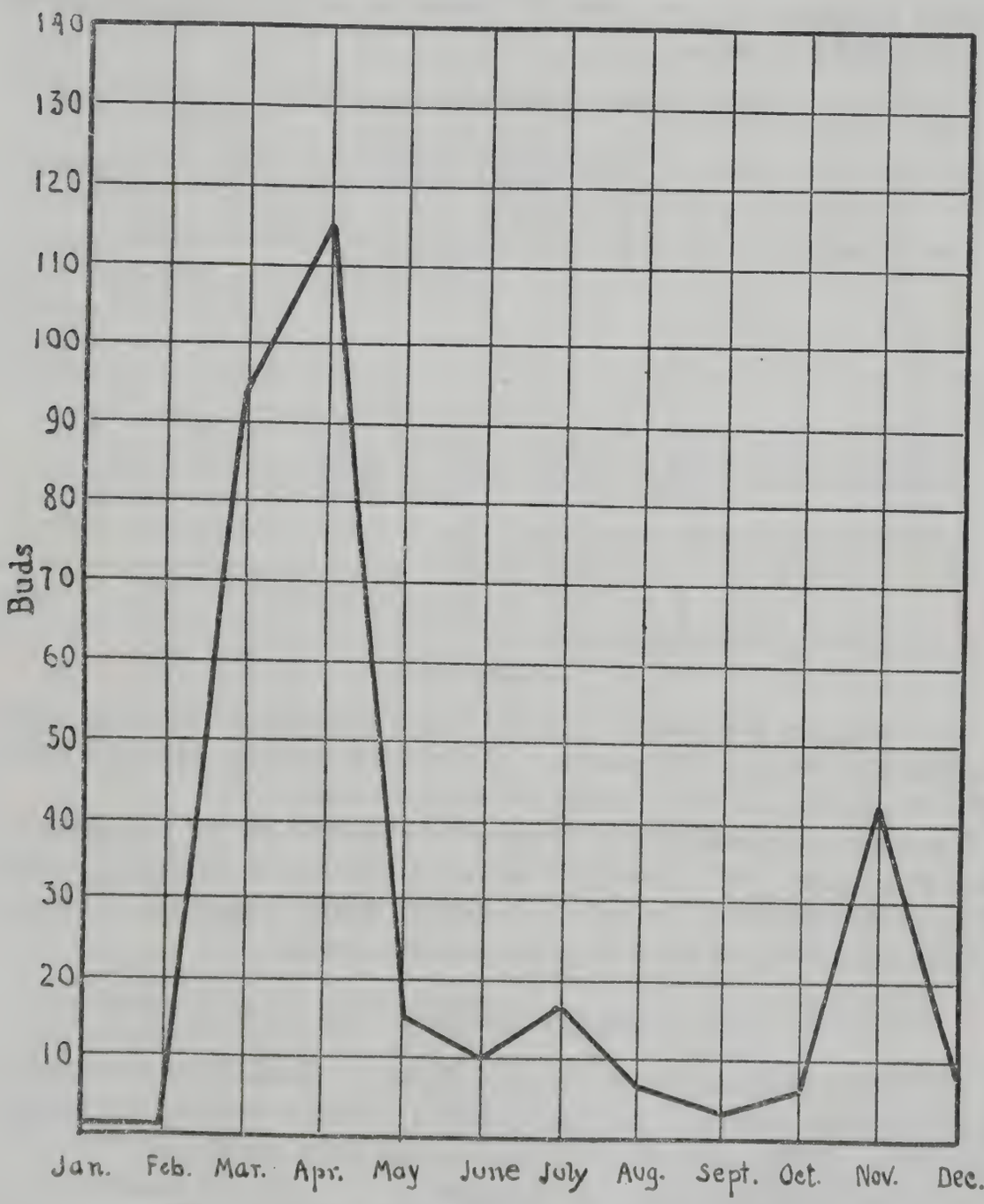

FIG. 1.—Average monthly production of lemon buds during the year.

The appearance of large numbers of fruit buds in March and April is undoubtedly related to the greater activity of the tree, following its slower winter growth. The secondary maximum in November, following the last growth cycle of the tree for the season, is not so easy of explanation. It might be assumed that the appearance of buds at this time was a reaction to the large supply of elaborated food material in the tree

and that the tree responded by putting forth fruit buds, while hindered by climatic conditions from producing vegetative growth.

In view of the fact that there are two periods of the year in which there is a maximum production of buds, it is logical to expect that the coefficient of correlation between time and number of buds would be negative, since the maximum production is in the early part of the year.

Table II shows the array for the figures representing the production of new buds by months.

TABLE II.—*Correlation between new buds and the month in which they appear*

Mean number of new buds per 50 twigs by months	Months, beginning with March												
	1	2	3	4	5	6	7	8	9	10	11	12	
1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1	2
4	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	1
6	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	1
7	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	1
8	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	1
10	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	1
15	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
16	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	1
42	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	1
95	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
115	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
	1	1	1	1	1	1	1	1	1	1	1	1	12

$r = -0.650 \pm 0.112$

The coefficient is strongly negative and is in harmony with the observations, showing that the numbers of new buds decrease after the spring months, though not in a strictly linear regression.

A certain synchronism was frequently observed in the production of new fruit buds. If a branch blossomed heavily in March, it would blossom heavily again in July. A branch which blossomed heavily in August was likely to blossom heavily in November.

THE SIZE AND PRODUCTIVENESS OF THE INFLORESCENCES

The lemon flowers occur singly or in clusters. During the rapid growth of spring large inflorescences are common; at other seasons the inflorescences are smaller and many of them possess only one flower.

Statistical studies were made to ascertain the range of variability and the productiveness of the inflorescences on these particular lemon trees. Data on 1,363 inflorescences which appeared during the course of the observations were examined. The number of flowers per inflorescence ranged from 1 to 28. The relative frequency of the inflorescences in relation to the number of buds per inflorescence is shown in Table III. The data show that the greatest frequency occurred in the class of inflorescences which bore a single bud and that the frequencies decreased quite

uniformly as the number of buds per inflorescence increased in succeeding classes. From these data the following constants were calculated:

Mean number of buds per inflorescence = 4.784 ± 0.071.

Standard deviation = 3.916 ± 0.050.

Coefficient of variability = 81.86 ± 1.62.

An inspection of the figures shows several interesting relationships. The number of buds per inflorescence shows no tendency whatever to follow the normal curve of errors; therefore we may conclude that the number is not determined by pure chance, but, on the contrary, is fixed by some other influence. If the number of buds had been determined by purely casual factors, such as position on the tree, age of wood, or climatic conditions, we should be warranted in expecting a purely chance distribution. In a following paragraph it is shown that there is a correlation indicating that larger inflorescences occur in the spring months, but the coefficient expressing this correlation is not such that much emphasis can be laid upon it.

TABLE III.—*Frequency of inflorescences in relation to number of buds they produced*

Number of buds per inflorescence.	Number of inflorescences observed.	Number of buds per inflorescence.	Number of inflorescences observed.	Number of buds per inflorescence.	Number of inflorescences observed.	Number of buds per inflorescence.	Number of inflorescences observed.
1	239	9	51	17	7	25	2
2	216	10	37	18	4	26	1
3	173	11	18	19	2	27	1
4	168	12	20	20	0	28	2
5	134	13	18	21	0		
6	125	14	7	22	3		1,363
7	62	15	7	23	2		
8	53	16	11	24	0		

Since the distribution of the buds on the inflorescences departs so widely from that to be expected upon the basis of pure chance, it seems logical to assume that it is determined by factors which reside in the tree and not by external factors. In other words, the Lisbon lemon tree has an inherited tendency to produce few-flowered inflorescences which outweighs the effect of external influences.

The writer has found very few recorded studies upon this question, though it would seem worthy of study both from practical and theoretical standpoints. The frequency of distribution of the number of seeds in receptacles of the lotus (*Nelumbium luteum*) was found to agree very closely with that of a chance distribution.[1] It should be noted, however, that the two cases differ in the morphology of the organs in question. In lotus we are dealing with an organ developing from a compound ovary—that is, with one flower; but in the lemon inflorescence we are dealing with a short branch bearing flowers. It is possible that the

[1] PEARL, Raymond. VARIATION IN THE NUMBER OF SEEDS OF THE LOTUS. *In* Amer. Nat., v. 40, no. 479, p. 757–768, 4 fig., 1906.

number of seeds developed from a compound ovary is dependent upon a set of external, casual factors, such as amount or variability of pollen, conditions under which pollination occurs, and access of the mother plant to suitable supplies of nutriment. On the other hand, the number of flowers produced on an inflorescence may be more largely predetermined in the mother plant by such internal factors as those which determine the position and arrangement of leaves and others which act to produce generic and specific characters.

The study of the inflorescence may be carried a step further by attempting to determine whether the larger inflorescences were more characteristic of one season than of another. If so, it might show whether the size of the inflorescence is in any way influenced by seasonal conditions. Data for 403 inflorescences were available and represented a fair random sample as far as seasonable distribution is concerned. Table IV shows the correlation between the average number of new inflorescences on the seven trees and the average number of buds per inflorescence. It seemed more nearly correct to make this sort of correlation than one between months of the year and number of buds per inflorescence, since it eliminates irregular regression due to periodicity, leaving numbers of buds as the two factors for correlation.

TABLE IV.—*Correlation between monthly average size of inflorescence and numbers of inflorescences produced*

Average number of new inflorescences on seven trees per month	Average size of inflorescence (number of buds)												
	1.1	1.3	1.6	1.8	1.9	2.3	3.3	4.6	4.7	4.9	5.0	5.4	
2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	1
4	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
6	.....	.....	.....	1	.....	.....	.....	.....	1	.....	.....	.....	2
9	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1
18	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
33	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	1
40	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	1
44	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	1
59	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	1
170	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	1
	1	1	1	1	1	1	1	1	1	1	1	1	12

$r = 0.351 \pm 0.171$

The average number of new inflorescences per month on the seven trees ranged from 2 to 170; the average size by months ranged from 1.1 buds to 5.4 buds. The coefficient of correlation between these factors is 0.351 ± 0.171. Since the coefficient is only twice its probable error we must regard it as rather doubtful in indicating a correlation between these factors. It may be taken, however, to indicate that larger inflorescences were more abundant in seasons in which the number of new buds

was greatest—namely, in the spring months, or at other times at which the activity of the tree is at its height. This conclusion is in agreement with the repeated observation that thrifty trees most commonly bear lemons in clusters.

The next question to be investigated was one of considerable physiological interest: What is the correlation between the number of buds per inflorescence and the numbers of fruits matured per inflorescence?

A positive correlation approaching 1 is to be expected in case buds on all sizes of inflorescences have equal chances of development; a value much below this indicates that a bud on a larger inflorescence has a poorer chance. It is certain from the nature of the case that there must be some relationship between the two, since an inflorescence having only 1 flower could not produce more than 1 fruit, but an inflorescence possessing 20 flowers may or may not mature a proportional number of fruits. If we take the fruits whose history could be definitely ascertained and arrange them with regard to the size of the inflorescence from which they developed, we get the arrangement shown in Table V.

TABLE V.—*Correlation between numbers of buds and fruit on inflorescences of various sizes*

Buds per inflorescence	Fruits matured per inflorescence						
	0	1	2	3	4	5	
1	217	22	.....	.....	.....	.....	239
2	186	29	1	.....	.....	.....	216
3	144	27	2	.....	.....	.....	173
4	133	27	7	1	.....	.....	168
5	107	22	4	1	.....	.....	134
6	96	23	6	.....	.....	.....	125
7	50	4	5	1	1	1	62
8	44	7	1	1	.....	.....	53
9	38	8	5	.....	.....	.....	51
10	31	4	2	.....	.....	.....	37
11	12	5	1	.....	.....	.....	18
12	14	4	2	.....	.....	.....	20
13	14	3	1	.....	.....	.....	18
14	5	1	1	.....	.....	.....	7
15	5	.....	2	.....	.....	.....	7
16	5	2	4	.....	.....	.....	11
17	6	.....	.....	.....	1	.....	7
18	3	.....	1	.....	.....	.....	4
19	2	.....	.....	.....	.....	.....	2
22	3	.....	.....	.....	.....	.....	3
23	2	.....	.....	.....	.....	.....	2
25	2	.....	.....	.....	.....	.....	2
26	1	.....	.....	.....	.....	.....	1
27	1	.....	.....	.....	.....	.....	1
28	.....	.....	1	.....	1	.....	2
	1,121	188	46	4	3	1	1,363

$r = 0.178 \pm 0.017$

As determined from these figures, the coefficient of correlation is 0.178 ± 0.017, indicating a positive correlation between the size of the inflorescence and the number of fruits it brings to maturity. The small value of the coefficient, however, is worthy of note. It was previously mentioned that we should expect upon *a priori* grounds a definite relationship between flowers and fruits, especially in the case of the smaller inflorescences. It will therefore be in order to inquire whether the larger inflorescences are relatively as fruitful as the smaller.

In order to secure a measure of the relative fruitfulness of the inflorescences, I have used a formula which Harris [1] published several years ago. The correlation coefficient as here used is intended to measure the correlation between the number of fruits produced per inflorescence and the deviation of this number from its probable value, in case the number of fruits per inflorescence is in the same proportion to the number of buds per inflorescence as the total number of fruits to buds in the entire population. It is computed from the formula,

$$r_{xz} = \frac{r_{xy} - V_x/V_y}{\sqrt{1 - r^2_{xy} + (r_{xy} - V_x/V_y)}}$$

where x = buds and y = fruits per inflorescence, V_x and V_y are the coefficients of variability of the two characters, and z is to be read as "the deviation of the number of fruits per inflorescence from its probable value."

The value of this correlation as computed for the lemon inflorescence is $r_{xz} = 0.183 \pm 0.018$.

This negative value of r_{xz} is interpreted to mean that there is a distinct negative correlation between the size of the inflorescence and its power to develop its buds into fruits. In other words, a bud on one of the smaller inflorescences has a greater chance of becoming a mature fruit than a bud on one of the larger inflorescences. The competition between individual buds on larger inflorescences seems to be too severe to allow all to survive. It is not, therefore, probable that the larger inflorescences are able to mature proportionally larger numbers of buds.

Heinicke [2] has recently reported that the reverse relationship exists in the case of the apple trees he studied. His figures indicated that a higher percentage of flowers develop into fruits on spurs producing six flowers each than on spurs producing four or five flowers. Further data on this question are to be desired.

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[1] HARRIS, J. Arthur. THE CORRELATION BETWEEN A VARIABLE AND THE DEVIATION OF A DEPENDENT VARIABLE FROM ITS PROBABLE VALUE. *In* Biometrika, v. 6, pt. 4, p. 438–443. 1909.

——— CORRELATION IN THE INFLORESCENCE OF CELASTRUS SCANDENS. *In* Mo. Bot. Gard. 20th Ann. Rpt. p. 116–122. 1909.

[2] HEINICKE, Arthur J. FACTORS INFLUENCING THE ABSCISSION OF FLOWERS AND PARTIALLY DEVELOPED FRUITS OF THE APPLE (PYRUS MALUS L.). N. Y. Cornell Agr. Exp. Sta. Bul. 393, p. 41–114, illus. 1917. Bibliography, p. 112–114.

THE TIME REQUIRED FOR FRUIT TO DEVELOP FROM BLOSSOM TO MATURITY

Lemon fruits grow slowly in comparison with the fruits of most deciduous trees. About two months are usually required from the unfolding blossom until a lemon fruit reaches a diameter of ¼ inch. The time required for the fruit to reach a size of 2¼ inches (a desirable commercial size) varies according to conditions from 7 to 14 months. From the standpoint of the producer it is desirable to have the lemons reach mature size as soon as possible. A lemon which grows uniformly and rapidly is usually of superior quality. It reaches the size required for marketing without turning yellow to any appreciable extent; it develops the desired flavor after being artificially cured and withstands deteriorating influences during transportation and storage.

In the lemon the ovary begins to grow very soon after the perianth withers and falls off. The style adheres for some time after this, but eventually the stigma and a part of the style separate and fall away. "Time of maturity" is regarded as the time at which a lemon is harvested. This time is usually determined by one of two things, either the fruit has reached a diameter of 2¼ inches or it has lost all green color from its surface.

The records of 239 fruits were examined and the time at which they were set was ascertained. The observations on the time required for maturity are given in Tables VI and VII. It was found that the time required to mature individual lemons ranged from 7 to 14 months. The average time for all lemons in these records was 10.2 months. On account of the small number of lemons set in the months of November, December, January, and February, the figures for these months are omitted from Table VI, because of the inevitably larger error involved in averaging a few numbers. The reader will understand that fruit set in a given month was a bud in the preceding month. If this is borne in mind, there is no confusion in comparing Table VI with other tables in this paper.

TABLE VI.—*Average time required for growth of lemons to maturity according to the month in which fruit set*

Month in which fruit was set.	Number of fruits observed.	Mean time required for maturity. Number of months.	Month in which fruit was set.	Number of fruits observed.	Mean time required for maturity. Number of months.
March	8	10.1±0.47	July	41	9.3±0.18
April	43	10.3± .16	August	23	11.7± .23
May	70	9.8± .10	September	10	11.6± .38
June	20	9.8± .30	October	9	11.7± .39

The table shows that there was a variation of about 2½ months in the average time required to produce a lemon, depending upon the month in which it set. Lemons which were set in May, June, and July came

to maturity in minimum time, and those set in August, September, and October required the maximum time.

Further light on the relation of the time of maturity to the time of setting was afforded by determining their correlation coefficient. Table VII shows the data. February is denoted as the first month in the subject column, since active growth begins in that month; and January is the last month.

TABLE VII.—*Correlation between the month in which lemons set and the time required for maturity*

Months required for maturity	7	8	9	10	11	12	13	14	Totals.
1	1	.....	.....	4	.....	.....	.....	.....	5
2	1	1	2	.....	.....	4	.....	.....	8
3	.....	11	1	5	19	6	.....	1	43
4	6	3	13	32	10	5	.....	1	70
5	1	3	8	2	3	3	.....	1	20
6	3	18	2	6	6	4	2	.....	41
7	1	.....	2	2	2	8	7	1	23
8	.....	.....	1	2	2	1	3	1	10
9	.....	.....	.....	.....	3	6	.....	.....	9
10	.....	.....	.....	.....	3	3	.....	.....	6
11	.....	.....	1	.....	.....	.....	.....	.....	1
12	.....	.....	1	.....	2	.....	.....	.....	3
	13	36	31	53	49	40	12	5	239

$r = 0.138 \pm 0.043$

The correlation coefficient denotes a positive relationship between the two factors, though its magnitude is not sufficient to warrant much emphasis. We can conclude that the season at which fruit is set influences, but does not absolutely determine, the length of time which will be required for maturity. Thus, fruit set in May had a range in time of maturing from 7 to 14 months, although about half the fruit required 10 months.

THE RATIO BETWEEN BUDS AND FRUIT WHICH REACHED MATURITY

The lemon may develop without pollination of the flower, therefore the proportion of fruit to buds may be expected to indicate the productiveness of the tree without entire dependence upon the chance of pollination as already intimated; however, the productiveness of the tree is greatly influenced by various environmental factors, especially by meteorological factors. Soil environment, as influenced by the application of fertilizers or water, affects fruit production; but its influence is not so abrupt and does not make itself quite so conspicuous as the former complex of factors.

An examination of these records may be of interest as an indication of what happens under good commercial culture. These trees stood in

a large plantation, receiving regular orchard treatment during the time observations were being made, and exposed to the vicissitudes which beset the commercial orchards. The effects of winter cold and of summer heat are plainly visible at places in the records, yet there is no reason to expect that the average lemon tree may escape the vicissitudes which befell these. The figures showing the proportion of buds which develop into mature fruit were based only upon observations made early enough to allow fruit to mature before the close of the observations.

Several definite stages in the development of fruit have been recognized in making this study. They are as follows: (1) the plump bud just ready to open; (2) the flower; (3) the first stage of the fruit at which the corolla has fallen but the style is still attached, designated for convenience "style attached"; (4) the young fruit having a diameter of ¼ inch and having lost the apical portion of the style; (5) the fruit having a diameter of 2¼ inches, ready to pick.

The individual histories of a random sample of lemon buds were followed from stage to stage to see what proportion survived and to locate, approximately, the time of heavy mortalities. A sample of 4,440 buds which appeared during the observation period was chosen. Their developmental history is shown in Table VIII.

TABLE VIII.—*Ratio of lemon buds to fruits which reached various stages*

Stage of development.	Number observed.	Per cent.
Buds ready to open	4,440	100.00
Styles attached	2,308	51.98
Fruit ¼ inch diameter	964	21.71
Fruit mature	294	6.62

These figures show that there is a large mortality between the young buds and the mature fruit, and that the mortality seems to increase with the age of the fruit. It should be stated, however, that losses were comparatively small after the fruit had reached a diameter of 1 inch. There is no reason, however, to regard the losses of fruit on these trees as abnormally high, since the crops produced on these trees were above the average for this district.

The effect of seasonal conditions upon the survival of young fruits seemed worthy of study in determining the ratio between buds and fruit. Since the time at which the fruit sets is the time at which it begins to grow, calculations were begun with the stage designated "style attached." Data were available for 2,453 fruits of this size which had the chance of developing into mature fruit during the time observations were made. Records were taken of the numbers of fruits reaching this stage in each month and of the numbers which matured from each of the several samples of "style attached" fruits.

TABLE IX.—*Relation of the survival of fruits to the months of the year in which they were set*

Month.	Number of "style-attached" fruits observed.	Mature fruits produced.	
		Number.	*Per cent.*
January	262	4	1.5
February	113	2	1.8
March	185	25	13.5
April	51	21	41.2
May	501	106	21.2
June	215	24	11.2
July	189	57	30.2
August	232	19	8.2
September	168	25	15.0
October	106	2	1.9
November	191	16	8.4
December	240	2	.8
Total	2,453	303	.....

It is apparent from these figures that there is considerable variability in the chances of survival, depending upon the time of year at which fruit is set. Fruit set in September or October is liable to be killed by cold weather in January, or if set in May or June is liable to be killed by hot weather in June. That which was set in the winter months was repeatedly chilled by low temperatures at night and its vitality was probably lowered. Fruit set in April and July appears to have the best chance of survival, though these relations may vary from one year to another.

It will perhaps be more nearly correct to group the records by seasons, since conditions are not necessarily confined to months.

TABLE X.—*Relation of survival to season at which fruit was set*

Season.	Number of "style-attached" fruits observed.	Mature fruits produced.	
		Number.	*Per cent.*
Spring:			
March	737	152	20.6
April			
May			
Summer:			
June	636	100	15.8
July			
August			
Autumn:			
September	465	43	9.2
October			
November			
Winter:			
December	615	8	1.3
January			
February			

These figures show that, upon the trees observed, a fruit set in one of the spring months had the best chance of survival and of reaching maturity. The chance of reaching maturity diminished as the seasons advanced until the next spring. On the other trees or in other localities the chances of survival might be quite different.

SUMMARY

(1) A small group of Lisbon lemon trees was studied for two years to obtain data upon their fruiting habits. The trees stood in a large commercial orchard and received no special treatment during the time observations were being made.

(2) Approximately 66 per cent of the fruit buds appeared during March and April, 13 per cent appeared in November, 17 per cent appeared between April and November, and about 3 per cent appeared during the winter months.

(3) The distribution of buds on an inflorescence showed no tendency to follow the normal curve of errors. Few-flowered inflorescences predominated numerically over many-flowered inflorescences. A bud on a small inflorescence had a greater chance of developing into a mature fruit than one on a large inflorescence. The competition between individual buds on larger inflorescences seems to be too severe to allow all to survive.

(4) The time required for the fruit to reach maturity varied from 7 to 14 months, according to conditions. Fruit which was set in May, June, and July came soonest to maturity. The season at which fruit was set appeared to influence, but not wholly to determine, the time which was required for maturity.

(5) The records for 4,440 buds showed that 51.98 per cent set fruit, 21.71 per cent reached a diameter of ¼ inch, and 6.62 per cent reached the stage of maturity.

(6) A fruit set in the spring months had the best chance of survival and of reaching maturity. The chances of reaching maturity diminished as the season advanced.

ULTRA-MICROSCOPIC EXAMINATION OF DISPERSE COLLOIDS PRESENT IN BITUMINOUS ROAD MATERIALS

By E. C. E. Lord

Petrographer, Bureau of Public Roads, United States Department of Agriculture

INTRODUCTION

On a cursory examination of bituminous solutions by means of the ultra-microscope, varying amounts of finely divided solid material held permanently in suspension will invariably be found. In certain hard native asphalts highly dispersed mineral matter is present in large quantities, and the high adhesive properties of such asphalts have been attributed largely to the selective absorption exerted by these colloids on certain portions of the bitumen.[1] Further investigations have led Richardson to conclude that some bitumens were absorbed in larger quantities, and consequently had a greater colloid-carrying capacity than others, and that this variation was apparently in accordance with their viscosity and the general character of the particular bitumen.[2]

As a result of these investigations, it seemed desirable to develop a reliable method of ultra-microscopic analysis whereby the number of disperse colloidal particles could be determined accurately in any type of bitumen, thereby furnishing a ready means for comparing their colloidal capacities and at the same time establishing a possible method for estimating the relative value of this property from a road-making standpoint. The fact, however, should be emphasized that the present investigations were undertaken essentially to develop a method for counting colloidal particles in bituminous solutions, and that in drawing comparisons of the relative supporting values from the results obtained, the original consistency of the materials employed should receive due consideration.

METHODS OF ULTRA-MICROSCOPIC EXAMINATION

When examined under the ordinary microscope, the great bulk of the colloidal material common to bituminous solutions is invisible. Early investigations by Siedentopf and Zsigmondy [3] have shown that the resolving power of the microscope is very greatly increased when particles are viewed in a powerful light against a dark background. This illumination was obtained originally by allowing a beam of light to enter the cell through a narrow slit at right angles to the axis of the instrument,

[1] Richardson, Clifford. THE THEORY OF THE PERFECT SHEET ASPHALT SURFACE. *In* Jour. Indus. and Engin. Chem., v. 7, no. 6, p. 463–465. 1915.

[2] ———. IMPORTANCE OF THE RELATION OF SOLID SURFACES AND LIQUID FILMS IN SOME TYPES OF ENGINEERING CONSTRUCTION. *In* Sci. Amer. Sup., v. 83, no. 2152, p. 198–199. 1917. Printed also in separate form.

[3] Zsigmondy, Richard. ERKENNTNIS DER KOLLOIDE. 186 p. Jena, 1905.

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Key No. D-16

where part of the rays were deflected from the surface of the suspended particles into the microscope, thus rendering them self-luminous and clearly visible while the remainder of the field remained dark. A similar effect may be obtained by means of a substage parabaloid condenser, with central stop, whereby the outer rays from the beam of light entering the microscope from below are brought by a series of reflections to a short focus within the cell and are totally reflected from the lower surface of the cell cover, leaving the field dark as before. Particles whose indices of refraction vary from those of the inclosing liquid intercepting these oblique rays diffract a portion of the light into the microscope and become luminously visible as in the former case while the remainder of the field is perfectly dark.[1]

In order to avoid loss of light through refraction of the rays issuing from the condenser, an immersion liquid, such as cedar oil or glycerin, should be employed between it and the cell containing the liquid under examination. This parabaloid illuminator is interchangeable with the substage Abbe condensor of the ordinary microscope and was consequently found most convenient for the present investigations. The microscope selected was provided with an accurately calibrated micrometer screw for vertical measurements and a mechanical stage for lateral orientation. A diamond point object marker with circular movement graduated to millimeters and insertible in the revolving nosepiece of the microscope will also prove a useful accessory. Light was furnished by a special arc lamp run on either direct or alternating current and regulated by a rheostat of 4.5 ampere capacity. Before entering the microscope the light was passed through a cooling solution, acting as a ray filter, of 10 mgm. diamine green dissolved in 1 liter of distilled water. A photograph of the microscope with arc light and ray filter used is shown in Plate 19, A.

The ordinary lens system of the microscope consisted of eyepieces ×7.5 and ×12.5 and objective 3, 4, and 16 mm., giving linear magnifications of 50 to 740 diameters at a tube length of 160 mm., while the best combination for counting was obtained with eyepiece ×7.5 and objective 4 mm., magnifying 320 diameters.

A counting device was inserted in the focal plane of this eyepiece, consisting of a cross-line micrometer scale with ground glass border divided into 25 square areas each side of which measured 1.25 mm. and corresponded exactly to 0.05 mm. of a stage micrometer at a tube length of 166 mm. With this micrometer the areal dimensions of any liquid under examination could be accurately determined, while the vertical element was obtained by means of the micrometer screw recording an interval of 0.00254 mm.[2]

[1] BURTON, E. F. THE PHYSICAL PROPERTIES OF COLLOIDAL SOLUTIONS. p. 46–47. London and New York. 1916.

[2] This micrometer was calibrated against that of a standard Fuess microscope registering a minimum vertical interval of 0.001 mm.

PREPARATION OF THE ULTRA-MICROSCOPE CELL

In order to carry out a quantitative analysis of bituminous solutions it was found necessary to employ a cell of minimum capacity that might be readily cleaned and hermetically sealed to prevent the escape of the volatile solvent. Efforts were made to utilize a container constructed on the principle of the Zeiss haemocytometer, as employed by Burton and Perrin in their examination of colloidal water solutions (hydrosols),[1] but it was found that all types of cement used in constructing this cell were attacked by the benzol solution and, furthermore, that the rulings on the bottom of the cell when filled with the solution were almost invisible under the microscope.[2]

In order to overcome the above-mentioned difficulties, it was found necessary to excavate a suitable cavity in the object glass itself, thereby doing away entirely with the superimposed glass plates of the haemocytometer slide. The object glass selected was as free as possible from air bubbles and other inclusions and had perfectly smooth plane surfaces and a thickness varying from 1.25 mm. to 1.75 mm. to assure a proper focus within the cell of rays from the dark field illuminator. he excavation was carried out by means of a stationary upright drill provided with a pointed vulcanized fiber cylinder having a flat grinding surface about 2 mm. in diameter. The drill was run by an electric motor at 1,800 revolutions per minute, using coarse emery mixed with a little heavy lubricating oil as an abrasive. In operating the drill great care was taken to apply a moderate uniform pressure, and the glass plate was protected from sudden strain by a folded towel or felt cushion placed beneath it. After grinding for one or two minutes the drill was removed and the cavity examined. In general, the central portion was found to be essentially flat and surrounded by deeper circular grooves, produced by the larger fragments of emery becoming lodged in the drill during the process of grinding.

From this stage in the operation the grinding was carried on by means of an electrically driven, flexible shaft drill constructed on the principle of the dental drill and using volcanic ash or ground pumice with water as an abrasive. This drill, operating at a speed of 1,540 revolutions per minute, was provided with a grinding point of vulcanized rubber or belata gum which also proved very effective in polishing the cell. The polishing was begun with diatomaceous earth and water and

[1] BURTON, E. F. op. cit., p. 118–120.

PERRIN, Jean. MOUVEMENT BROWNIEN ET RÉALITÉ MOLÉCULAIRE. *In* Ann. Chim. et Phys., s. 8, t. 18, p. 40–42. 1909.

[2] The indices of refraction for ordinary light flint glass and benzol at 21.5° C. are 1.5710 (D) and 1.5304 (H), respectively. SMITHSONIAN PHYSICAL TABLES, ed. 6, p. 184, 192. Washington, D. C., 1914. (Smithsn. Misc. Collect., v. 63, no. 6.) Hence light passing through glass and meeting etched lines on a cell bottom mounted in benzol are but slightly diffracted and consequently appear indistinct under ultra-microscopic illumination while plainly visible when viewed in water or air.

was continued with a mixture of freshly precipitated calcium carbonate and magnesia hydrate until a microscopically smooth and essentially flat surface was obtained. Finally, in the center of the cell a circle 1 mm. in diameter was inscribed with the diamond-point marker of the microscope in order to limit the field of observation. A diagram of the slide, with 2½ mm. cell (A) containing circular area (B) drawn to natural scale, is shown in figure 1, where the depth is indicated as lying between 0.044 and 0.143 mm. These values were determined as accurately as

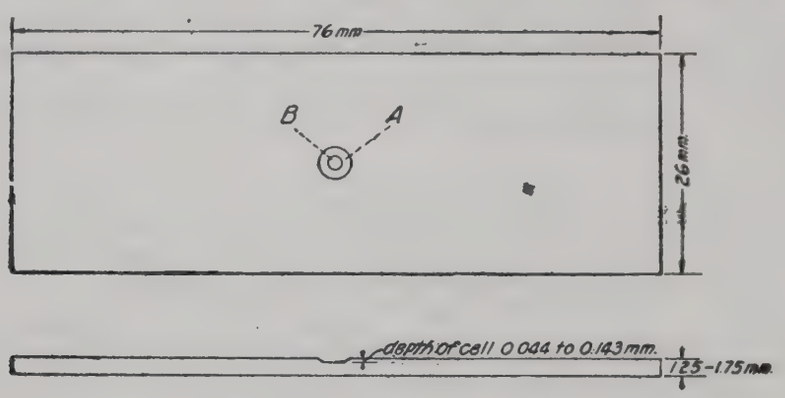

76 mm
B
A
26 mm
depth of cell 0.044 to 0.143 mm.
1.25–1.75 mm
A is saucer-shaped cell 2½ mm. in diameter.
B is circular field 1 mm. in diameter in center of cell floor.

FIG. 1.—Glass slide with ultra-microscope cell drawn to natural scale.

possible for each cell by means of a strain dial recording intervals of 0.0001 inch and were checked with the microscope micrometer under a magnification of 740 diameters. A blunted, highly polished needle point inserted in the vertical arm of the dial enabled readings to be taken at different points within the cell, and the average of these readings was compared with that of an equal number taken around the cell from without. The results of these measurements for a number of cells constructed in the manner outlined above, together with microscopic check determinations, inclosed in parentheses, are given in the following table:

TABLE I.—*Depth of ultra-microscope cells determined by strain dial*

Slide number.	Depth in mm.	Thickness of slide in mm.	Maximum variation in depth in mm.	Maximum variation in thickness in mm.	Percentage of variation in depth.	Percentage of variation in thickness of slide.
1	0.1209 [a] (.1220)	1.5316	0.0058	0.0061	4.83	0.40
2	.1321	1.5382	.0160	.0101	12.10	.66
3	.0434 (.0423)	1.7374	.0036	.0096	8.30	.55
4	.1432 (.1450)	1.6103	.0094	.0046	6.50	.28
5	.0795 (.0790)	1.4880	.0071	.0053	9.20	.36
6	.0892 (.0890)	1.6812	.0043	.0061	4.82	.36
Average	.1014	1.5978	.0077	.0070	7.53	.43

[a] Determined by microscope micrometer.

In comparing these values it will be noted that the average depth of all cells is but slightly in excess of 0.10 mm., while the maximum variation in depth and in the thickness of slide is approximately the same (0.007 mm.), indicating a cell floor closely approaching a true plane.

Dial measurements also were undertaken to determine the depth of cell, including cover glass after mounting in the asphaltic oil solution employed in counting (see below) and in air to form an estimate of the relative thickness of the liquid film between cover glass and slide beyond the cell area. In every case lesser values were obtained for cells mounted in this solution than in air, indicating a more perfect contact through the release of atmospheric pressure and the adhesive character of the bitumen.

PREPARATION OF SOLUTIONS

Before describing the method of counting colloidal particles employed in this investigation, it will be found desirable to outline briefly the general character of bituminous solutions containing colloidal matter and the manner in which these solutions have been prepared for microscopic analysis.

When viewed under the ultra-microscope the colloidal portion of the solution will appear as a mass of very finely divided and more or less widely dispersed particles undergoing a constant and, under certain conditions, perpetual movement (Brownian movement). This movement has been ascribed to the molecular energy of the suspending liquid and may be regarded as a function of the size of the particles and their degree of dispersion which, in turn, is limited by the viscosity of the solution.[1] In order, therefore, to count successfully these suspended particles it was found necessary either to retard their movement by suitable concentration of solution or to increase it by dilution to such a degree that they settled out within the cell inclosure.[2] In the latter case, however, it frequently happened that the particles were in part resorbed on exposure to light, thus destroying the accuracy of the count. To assure concordant results, therefore, the particles were always counted in a somewhat viscous solution of colloid-free asphaltic oil to which a definite amount of paraffin had been added. This solution was prepared by fluxing 2.5 gm. Mexican oil asphalt (penetration 148) with 0.5 gm. crystalline paraffin and diluting to 100 cc. with benzol containing 10 per cent alcohol. This was then evaporated to constant weight on the water bath, brought to original consistency with benzol and passed through an alundum tube or filter tube clogged with macerated filter paper until approximately all suspended matter had been removed.

[1] OSTWALD, Wolfgang. DIE WELT DER VERNACHLÄSSIGTEN DIMENSIONEN. p. 34–35. Dresden and Leipzig, 1915.

[2] The solvent used in these investigations was c. p. benzol, since carbon bisulphid was found to contain an appreciable quantity of colloidal sulphur.

When properly prepared, the diluting solution should contain not more than 10 particles to a ¼ mm. square field in cell No. 3, at a magnification of 320 diameters. A definite portion of the solution to be examined was introduced into this standard dilutant after the coarser mineral matter had been removed. That was accomplished by dissolving 1 gm. of the original sample in 50 cc. benzol in a stoppered centrifuge tube, allowing the solution to stand overnight (17 hours) and centrifuging for 1 hour at a speed of 800 revolutions per minute. A small portion of this solution was then drawn off from the tube at a depth of 10 mm., and 1 cmm. transferred to a glass-stoppered graduate and brought up to 10 cc. with the paraffin oil dilutant. By this means a dilution of 1 to 5,000 of the colloids present in the original sample was obtained. This was found to be sufficient in most cases, but in certain bitumens where the dispersed mineral matter was in a state of extreme subdivision a further dilution of 1 to 50,000 was necessary before the colloid particles could be conveniently counted.

METHOD OF COUNTING COLLOIDAL PARTICLES

The samples of bitumens selected for examination were obtained from the commoner types of road material, ranging from hard native asphalt to lighter oils and containing varying amounts of colloidal matter. After having been subjected to the preliminary treatment mentioned above, one or two drops of the properly diluted solution were rapidly transferred from the 10-cc. graduate to the cell by means of a 1-cc. pipette and covered immediately by a 18-mm. cover glass, using a bluntly pointed wooden rod to expel all excess liquid and assure a perfect contact between slide and cover glass. After the excess solution had hardened sufficiently by evaporation and the slide beyond the cell limit appeared perfectly clear and colorless, the cover glass was sealed with a 30 per cent solution of boiled Canada balsam in ether applied with a hair-line paintbrush (No. 0). A photomicrograph of a part of the mounted cell with cross-line micrometer scale magnified 320 diameters is shown in Plate 19, B.

In order to obtain consistent results, the cell and cover glass should be microscopically clean before mounting and the dilutant examined from time to time to allow for corrections in the final results.[1] When properly mounted the cell should be free from air bubbles and the colloidal particles should appear under the microscope evenly distributed and in constant, though restricted, motion. In correct focus these particles were clearly defined as brilliant points of light against a dark background, but a change of focus resulted in the development

[1] The cleaning was accomplished by first boiling slide and cover glass in concentrated sulphuric acid, then rinsing in water, alcohol, and benzol, drying with soft cotton or silk cloth, and rubbing with optical tissue paper until thoroughly clean.

of concentric halos or diffraction rims around each particle that detracted greatly from the definition of the images. In order to overcome this so far as possible, it was found necessary to employ cells below 0.10 mm. in depth, having a capacity less than 0.10 cmm. (Table I, No. 3, 5, and 6). Counts were made of all particles in suspension as well as those that might have settled out on the cell floor or become attached to the cover glass during the process of counting. The area examined was taken from within the central millimeter circle of the cell and represented exactly one-fourth of 1 square millimeter (equivalent to four fields of the cross-line eyepiece micrometer at a magnification of 320 diameters), while the volume of liquid was obtained from this area and the depth of cell employed (0.043–0.089 mm.). The number of particles counted in each of the 25 square subdivisions of the micrometer through the entire depth of liquid was recorded, and from the average of four such determinations the value for 1 cmm. of solution and 1 gm. of bitumen was computed.

The results of the analyses were recorded on a special form which, in addition to the data indicated above, contained information regarding the physical properties of the bitumen, together with the relative size and distribution of the colloidal particles. In general, it may be stated that these particles varied in size from submicrons having a minimum diameter of about 15 $\mu\mu$ (0.000015 mm.) to particles within the visibility of the ordinary microscope (above 0.25 μ=0.00025 mm.).[1] These dimensions may be determined by direct microscopic measurement or they may be calculated by dividing the total volume of particles contained in a definite quantity of solution by the number of particles found where the volume represents the weight of the particles divided by their specific gravity. The quotient thus obtained will equal the volume of one particle (x). Assuming the particles to be spheres of diameter a,

$$\text{Then } \frac{4}{3}\pi a^3 = x$$

$$a = \sqrt[3]{\frac{x}{\frac{4}{3}\pi}}$$

RESULTS OF THE ULTRA-MICROSCOPIC EXAMINATION OF BITUMINOUS SOLUTIONS

In order to standardize the method of ultra-microscopic analysis outlined above, a number of determinations were made of various colloidal materials contained in different types of bitumens. The results of these determinations are shown in Table II.

[1] ZSIGMONDY, Richard. OP. CIT., p. 88b–97.

TABLE II.—*Ultra-microscopic counts of colloidal particles in bituminous solutions*

Sample No.	Cell No.	Depth of cell in mm.	Bitumens used.	Consistency before treatment.		Consistency after treatment.	Number of particles counted per cmm. at 1:5000 dilution.	Average per cmm.	Maximum variation.	Per centage of variation.	Supporting value.
				Penetration.	Specific viscosity.	Penetration.					
1	3	0.0444	Refined Trinidad asphalt	2			400,000				
2	3	.0444	do	2			405,584				
3	5	.0802	do	2			416,000	406,256	16,000	3.94	100.0
4	6	.0892	do	2			400,726				
5	6	.0892	do	2			408,968				
6	3	.0444	Refined Trinidad asphalt and clay				30,000				
7	3	.0444	do				32,000				
8	5	.0802	do				29,120	30,529	2,880	9.43	7.5
9	6	.0892	do				31,031				
10	6	.0892	do				30,495				
11	3	.0444	Trinidad petroleum residuum and clay		105 at 25° C.	235	167,630				
12	5	.0802	do		do	235	169,260				
13	5	.0802	do		do	235	163,670	169,262	16,082	9.50	41.6
14	6	.0892	do		do	235	179,752				
15	1	.1370	do		do	235	166,000				
16	3	.0444	Mexican oil asphalt and clay	145		13	106,000				
17	5	.0802	do	145		13	106,786	108,113	4,020	3.21	26.6
18	6	.0892	do	145		13	109,417				
19	6	.0892	do	145		13	110,135				

TABLE II.—*Ultra-microscopic counts of colloidal particles in bituminous solutions*—Continued.

Sample No.	Cell No.	Depth of cell in mm.	Bitumens used.	Consistency before treatment.		Consistency after treatment.	Number of particles counted per cmm. at 1:5000 dilution.	Average per cmm.	Maximum variation.	Percentage of variation.	Supporting value.
				Penetration.	Specific viscosity.	Penetration.					
20	3	.0444	Mexican oil asphalt, 16 per cent $Cu.CO_3.Cu(OH)_2$	57	.....	8	2,892,000	2,858,000	92.000	3.29	703.5
21	3	.0444	.....do.....	57	.....	8	2.800,000				
22	3	.0444	.....do.....	57	.....	8	2,892,000				
23	3	.0444	Trinidad petroleum residuum, 16⅔ per cent $CuCO_3.Cu(OH)_2$.	.....	226 at 100° C.....	18	1,089,900	1,085,950	7,900	.73	267.3
24	5	.0802	.....do.....	.....	.....do.....	18	1,082,000				
25	3	.0444	Mexican oil asphalt, 10 per cent $CuCO_3.Cu(OH)_2$	57	.....	10	1,033,200	1,012,800	108,000	11.25	249.3
26	5	.0802	.....do.....	57	.....	10	990,000				
27	3	.0444	.....do.....	57	.....	10	960,000				
28	5	.0802	.....do.....	57	.....	10	1,068,000				
29	3	.0444	Trinidad petroleum residuum, 10 per cent $CuCO_3.Cu(OH)_2$	.....	226 at 100° C.....	25	805,000	785,500	39,000	5.09	193.3
30	5	.0802	.....do.....	.....	.....do.....	25	766,000				
31	3	.0444	Mexican oil asphalt, 20 per cent $Pb(C_2H_3O_2)_2{-}3H_2O$	250	.....	70	284,800	262,400	44,800	18.7	64.6
32	5	.0802	.....do.....	250	.....	70	240,000				
33	3	.0444	Mexican oil asphalt, 10 per cent $CuSO_4$	57	.....	16	209,000	204,160	18,000	9.2	50.2
34	6	.0892	.....do.....	57	.....	16	214,000				
35	6	.0892	.....do.....	57	.....	16	201,800				
36	3	.0444	.....do.....	57	.....	16	200,000				
37	5	.0802	.....do.....	57	.....	16	196,000				
38	3	.0444	Gilsenite, 10 per cent $CuCO_3.Cu(OH)_2$	.....	.....	.....	101,442	100,721	1,442	1.44	24.8
39	3	.0444	.....do.....	.....	.....	.....	100,000				
40	3	.0444	Water gas tar, 20 per cent $CuCO_3.Cu(OH)$	.....	(a)	21	20,000	20,455	2,536	12.4	5.0
41	3	.0444	.....do.....	.....	.....	21	19,420				
42	5	.0802	.....do.....	.....	.....	21	21,956				
43	3	.0444	Mexican oil asphalt, 10 per cent $Fe.SO_4$	57	.....	28	20,000	18,667	2,000	10.7	4.6
44	3	.0444	.....do.....	57	.....	28	18,000				
45	5	.0802	.....do.....	57	.....	28	18,000				
46	3	.0444	Mexican oil asphalt, 10 per cent $ZuSO_4$	57	.....	16	4,248	4,459	422	9.46	1.1
47	5	.0801	.....do.....	57	.....	16	4,670				

(a) Float test=153″ at 32° C.

It will be noted that duplicate check counts were made generally in cells of varying capacity, giving the maximum numerical and percentage variation and indicating as well the supporting value or colloidal capacity of each type of bitumen, based on that of refined Trinidad asphalt considered as 100. All results were computed on a basis of 1 to 5,000 dilution of the original colloidal portion of the sample. (See p. 175.) These bitmuens have been separated into groups containing clay as colloidal material (No. 1–19) and into others in which this mineral matter was replaced by carbonates, sulphates and acetates of copper, iron, zinc, and lead (No. 20–47). The samples included in the first class, except untreated refined Trinidad asphalt (No. 1–5), were prepared by incorporating 33 per cent sandy clay in each type of bitumen by Richardson's method of heating an aqueous emulsion of clay and bitumen until all moisture and gas had been expelled.[1] In the case of refined Trinidad asphalt and clay (No. 6–10) all insoluble mineral and organic matter originally present in the bitumen was removed before emulsifying by dissolving in benzol and adding about 2 per cent shellac dissolved in alcohol and evaporating to constant weight, redissolving in benzol and filtering through an alundum tube until the solution was essentially void of colloidal particles.

In the second group of bitumens (No. 20–47) the salts were introduced in an anhydrous condition and the mixtures were heated to about 170° C. under constant stirring until all evolution of gas had ceased. On examining solutions of this kind under the ultra-microscope it was found that the copper carbonate salts had been largely reduced to red cuprous oxid, accompanied by an enormous colloidal dispersion (No. 20–30), while with the remaining salts the reduction had been much less complete (No. 31–39) or entirely lacking (No. 40–47), and the development of colloids correspondingly less. It may be stated, therefore, that the colloidal capacity of the second group of materials taken as a whole was dependent largely upon the degree of chemical reaction between the bitumen and the salts employed, while in the first group this supporting value was related more directly to the physical character of the bitumen.

A comparison of the duplicate counts recorded in columns 3 and 4 of the table indicated that a maximum variation of less than 10 per cent was attained in samples of the first group (No. 1–19), while in the second group (No. 20–47) the results were, on the whole, less concordant, owing largely to the greater dispersion of colloidal matter.

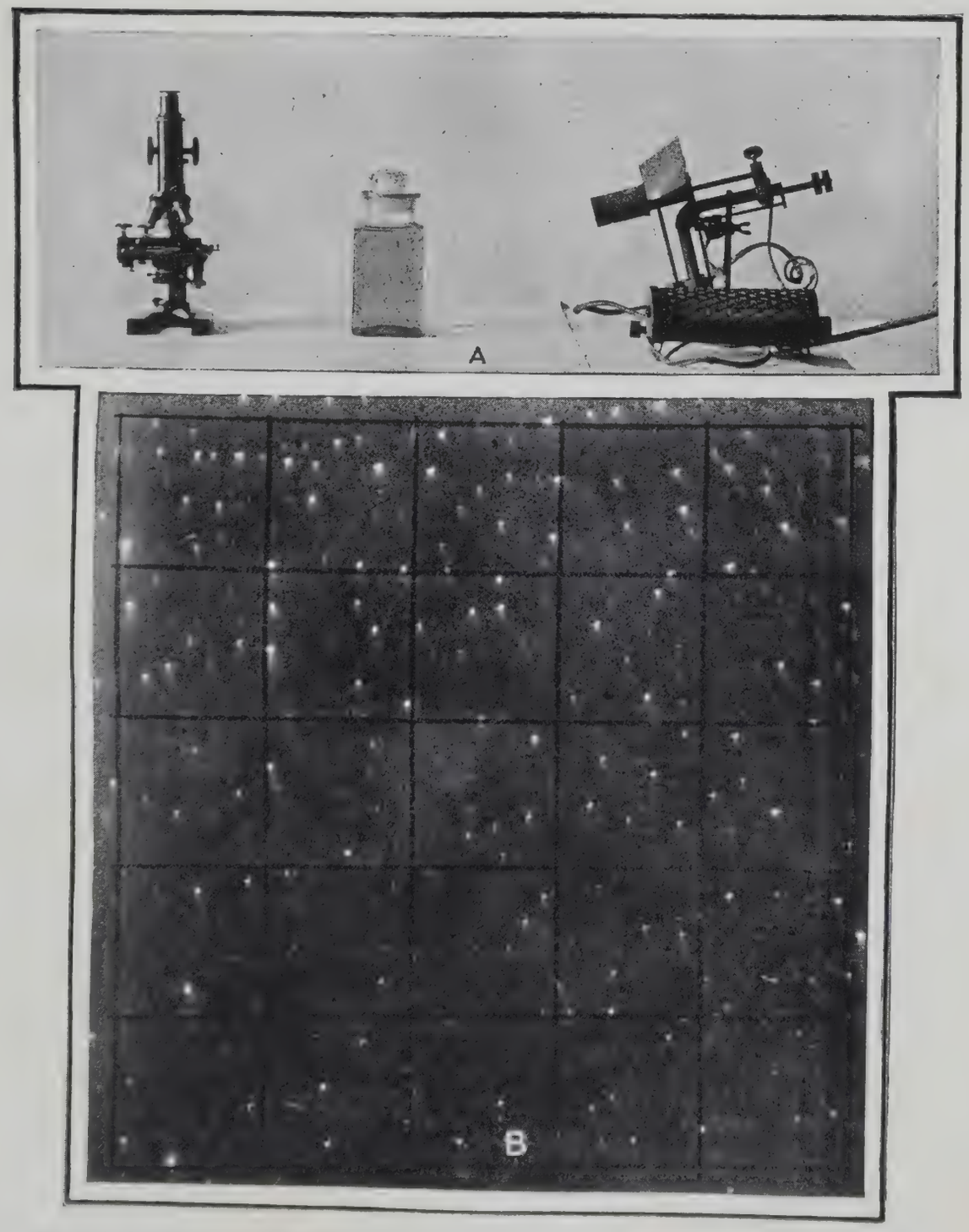
In conclusion, it may be stated that the accuracy of this method for counting colloidal particles in bituminous solutions depends chiefly upon accuracy in construction and calibration of the cell employed, as well as upon the proper consistency and optical purity of the supporting liquid.

[1] Richardson, Clifford. 1917. op. cit.

PLATE 19

A.—Microscope with ray filter and arc lamp for dark field illumination.

B.—Photomicrograph of cross-line micrometer scale, showing colloids in dark field. ×320. Taken by E. A. Shuster, jr., Photographic Laboratory, United States Geological Survey.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DERRIS AS AN INSECTICIDE

By N. E. McINDOO, *Insect Physiologist, Deciduous Fruit Insect Investigations, Bureau of Entomology,* A. F. SIEVERS, *Chemical Biologist, Drug-Plant and Poisonous-Plant Investigations, Bureau of Plant Industry,* and W. S. ABBOTT,[1] *Scientific Assistant, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The investigation of the possibilities of Derris as an insecticide is a continuation of the cooperative work inaugurated by the Bureaus of Entomology and Plant Industry, and the most important results pertaining to the study of Derris are discussed in this paper. There are now on the market several standard insecticides: Arsenicals, used as stomach poisons; nicotine solution, used as a contact insecticide; pyrethrum powder, employed as a dusting powder; and soaps, lime sulphur, oil sprays, etc. Not one of these acts both as a stomach poison and a contact insecticide. The following pages will show how well Derris acts in both of these ways.

In a search through the vegetable kingdom for plants possessing toxic principles with a view toward utilizing them as insecticide material, attention was directed to the large class of plants which are used extensively in the tropics as fish poisons. There are many hundreds of these plants, included in several families, and their habitat extends over practically the entire Tropics. That many of them belonging to particular families and genera display a remarkably toxicity to fish has long been known, and probably for ages the natives of the Tropics have used some of these plants as a means of catching fish.

While a plant toxic to fish need not necessarily be poisonous to insects, nevertheless, some of the fish poisons have already been recommended and used in the Orient as insecticides. If the fish poisons prove to be efficient insecticides, their practical utilization is at once suggested, because many of them are known to be very abundant in the Tropics. The present investigation deals with six or seven species, all belonging to the same genus, which is widely known as Derris.[2] Of these species only *Derris elliptica* Benth. seems to have been used widely as a means for catching fish; it is regarded as a powerful fish poison.

[1] A portion of the experimental part of this investigation was performed at the Insecticide Board's testing laboratory, located at Vienna, Va., by W. S. Abbott and E. W. Scott, Entomologist, Enforcement Insecticide Act, under the direction of the latter.

[2] Although this genus has commonly been known as Derris, the rules of botanical priority require the use of the name Deguelia of Aublet. Of the six species mentioned, the following have received names under Deguelia: *Deguelia elliptica* (Wall.) Taub. [*Derris elliptica* (Wall.) Benth.]; *Deguelia robusta* (Roxb.) Taub. [*Derris robusta* (Roxb.) Benth.]; *Deguelia timorensis* (DC.) Taub. [*Derris scandens* (Roxb.) Benth.] *Deguelia uliginosa* (DC.) Baill.; [*Derri. uliginosa* (DC.) Benth.]. S. F. BLAKE.

The material available for the present study was secured in most cases from various agricultural and botanical agencies through the Office of Foreign Seed and Plant Introduction, United States Department of Agriculture. The following is a list of the material used and the sources from which and through which it was secured: Powdered roots of a Derris species, most likely *Derris elliptica* Benth., from the open market where it is sold as an insecticide; roots of *D. elliptica*, called "tuba" or "toeba" in the Dutch East Indies, from the 's Lands Plantentuin, Buitenzorg, Java; stems of *D. uliginosa* Benth., from Mr. C. H. Knowles, Suava, Fiji Islands; stems of *D. koolgibberah*[1] Baill., and of *D. oligosperma*,[1] from the director of the Botanical Gardens at Brisbane, Queensland, Australia; roots of *D. scandens* Benth.; and stems and roots of *D. robusta* Benth., from the director of the Botanical Survey of Sibpur, Calcutta, India.

HISTORICAL REVIEW

The genus Derris, belonging to the family Papilionaceae, tribe Dalbergieae and subtribe Lonchoecarpinae, is practically native throughout the Tropics, but is far more abundant in the Old World than in tropical America. Its members are climbing shrubs, having trunks 3 or 4 feet in height and about 4 inches in diameter; the trunks send out numerous long branches, which climb over the neighboring vegetation, and the tips of which hang freely downward.

Watt (*10, p. 80*)[2] describes Derris as a genus of arborescent climbers or trees, and states that the roots of *Derris elliptica* furnish a useful insecticide for gardening purposes. A number of other species are mentioned in the literature as being used for fish poisons, and in some cases reference is made also to their use as insecticides; but these cited cases seem to be no better than mere reports. Correspondents in the Philippine Islands and Java report that *D. elliptica* is probably the species most commonly used as a fish poison. In all cases, so far as known, only the roots are employed. It seems that the most widespread treatment is one in which the roots are buried in mud, brackish mud preferred, for a period of several weeks; then the roots are crushed and placed in water inhabited by fish. The roots of Derris, in all probability *D. elliptica*, are used as insecticides in the Dutch East Indies; and a correspondent reports that Derris is commonly used by the Chinese gardeners in the Malay Peninsula as an insecticide and that the parts of the plant used are sold by Chinese storekeepers. However, it is said that the poison loses its activity when the plant is dried.

Hooker (*5, p. 43*) reports on a specimen of *Derris elliptica*, obtained from Singapore where it is known as "tubah" and where it is used as an insecticide; the roots are steeped in water and the resulting decoction is said to be an efficient insecticide for garden purposes.

[1] No record of the publication of these specific names could be found.

[2] Reference is made by number (italic) to "Literature cited," p. 200.

Probably the first investigator to report on a chemical examination of *Derris elliptica* was Greshoff (*3*) in 1890. He found the most important constituent of the bark on the root to be a nitrogen-free, non-glucocidal resin which he called "derrid." He describes this resin, which he did not succeed in obtaining in crystalline form, as readily soluble in alcohol, ether, chloroform, and amyl alcohol, but soluble with difficulty in water and potassium hydroxid. The yield obtained from the whole root was 2.5 to 3 per cent. The resin was found to be extremely toxic to fish.

Dymock, Warden, and Hooper (*2, p. 471*) record that in India *Derris uliginosa* is used as an insecticide against larvæ of insects.

In 1892 Wray (*11*) worked on *Derris elliptica* and appears to have been unaware of Greshoff's paper, because for the resinous principle which he isolated from the root in an impure state and which he used in his experiments on fish he proposed the name "tubain." This substance is without question the same as Greshoff's "derrid," judging from its physical properties. The crushed roots when boiled in a retort with water yielded an opalescent distillate, the odors of which strongly resembled those from the roots. This distillate was found poisonous to fish.

In 1899 van Sillevoldt (*9*), working on *Derris elliptica*, reported on the extraction of Greshoff's "derrid." He used practically the same method of extraction as did Greshoff and describes the "derrid" obtained as a yellow, amorphous powder. In the impure "derrid" he found a crystallizable substance which was very insoluble in ether, by which means it could be separated from the soluble portion of the "derrid." He found the melting point of "derrid" to be near 73° C. and he describes it as being readily soluble in alcohol, ether, benzol, aceton, glacial acetic acid, acetic ether, carbon disulphid, and chloroform, and very insoluble in petroleum, ether, and water. Van Sillevoldt assigned the formula $C_{33}H_{30}O_{10}$ to "derrid."

In 1902 Power (*8*) investigated the stems of *Derris uliginosa*. His results led to the conclusion that the poisonous constituent of the plant is a resin, thus concurring in the views of Greshoff and van Sillevoldt. He noted further that this resin consists of two components, one being soluble in chloroform and highly toxic to fish, and the other insoluble in chloroform and inactive to fish.

In 1911 van Hasselt (*4*) investigated the physiological action of "derrid" on fish, frogs, mice, rabbits, and cats, and studied its effects on the blood, respiration, circulation, intestinal tract, and nervous system. From his experiments he concluded that "derrid" is a powerful poison, causing characteristic symptoms in all the animals treated, and that it kills by causing respiratory paralysis.

In 1916 Campbell (*1*) investigated the poisonous actions of *Derris elliptica*, and his work seems to be the most recent along this line. He tested the water and saline extracts of the roots on fish, mosquito larvæ,

tadpoles, toads, and monkeys. The following are the salient conclusions of his investigation: (1) Boiling does not destroy the toxic action of the sap; (2) roots kept three months in a cupboard retain their strength; (3) milky extract introduced into a fish's stomach is rapidly fatal; (4) tadpoles are fatally affected, but stronger extracts are required to kill them than to kill fish; (5) much stronger doses are required to kill mosquito larvæ than to kill either fish or tadpoles; (6) the extract from 1/50 gm. of the roots when injected subcutaneously is fatal to toads, and the extract from 1/12 gm. causes death when introduced into the stomach; and (7) when the extract from 2 gm. of the roots is injected subcutaneously or introduced into a monkey's stomach death results. Campbell further states (*p. 134–135*):

> From the results on different animals it is evident that the poison affects the more highly developed members of the animal kingdom more readily than it does the primitive members. This is only to be expected since its action concerns the brain and one particular part of this, namely, the medulla oblongata.
>
> It could be used to destroy mosquito larvæ, but it should be used in solutions not weaker than 1 in 1,000, that is just enough of the extract should be added to the pool to make the water cloudy.

METHODS OF PREPARING AND TESTING EXTRACTS FROM DERRIS MATERIALS

The many preliminary experiments performed indicate that Derris (probably *D. elliptica*) is promising as a contact insecticide and as a stomach poison but is of no practical use as a fumigant. The best methods of applying it—whether in the form of powder, suspended in water or in the form of extract mixed with water or with soap solution—now remain to be determined.

A vegetable insecticide is usually applied either in the form of fine powder or as a spray mixture. This mixture may consist of any one of the following four combinations: (1) Powder suspended in water; (2) aqueous extract of the material diluted with water; (3) a solution consisting of water and a small amount of a concentrated form of the active constituent; and (4) a small quantity of a concentrated form of the active principle suspended in water.

Since Derris material must be imported, only dried roots and stems may be secured for insecticidal purposes. As already stated under the historical review, the natives pound the roots of Derris into a pulp which they then throw into the water inhabited by fish. This allows the juices of the plant to mix freely with the water and is the simplest way of obtaining a water extract, but will water remove the toxic principle after the roots have become dry? The chief object of the investigation discussed under the preceding heading was to make a study of the different methods of extracting Derris and to determine the value of various solvents in order that a simple and economical method might be devised for obtaining the active principle and applying the extracts.

QUANTITATIVE EXTRACTIONS OF DERRIS AND PRELIMINARY TESTS OF EXTRACTS OBTAINED.

Five series of quantitative extractions were made as follows: In each series 20 gm. of fine powder of *Derris* sp. (probably *D. elliptica*) were exhausted successively with the following five solvents in the order named: First series, petroleum ether, ether, chloroform, alcohol, and water; second series, ether, chloroform, alcohol, water, and petroleum ether; third series, chloroform, alcohol, water, petroleum ether, and ether; fourth series, alcohol, water, petroleum ether, ether, and chloroform; and fifth series, water, petroleum ether, ether, chloroform, and alcohol. No heat was used in any of these extractions. Table I gives the percentages of extracts thus obtained. The sequence is shown by the letters a . . . a, b . . . b, c . . . c, etc., beginning with the first extraction in each case.

TABLE I.—*Successive quantitative extractions of Derris sp. with various solvents, starting with a different solvent for each series*

No. of extraction.	Solvents used.				
	Petroleum ether.	Ether.	Chloroform.	Alcohol.	Water.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
First	4.07 a	7.90 b	10.60 c	11.25 d	9.75 e
Second	4.55 e	4.90 a	.75 b	3.60 c	8.45 d
Third	.55 d	2.00 e	.20 a	3.30 b	5.00 c
Fourth	.50 c	.49 d	.50 e	2.59 a	5.05 b
Fifth	.10 b	.20 c	.20 d	1.10 e	10.80 a

Attention is called to the following points in the preceding table. From the first extractions it will be seen that petroleum ether is a poor solvent, while the other four may be called good ones; of these four, only alcohol and water can be regarded as economic solvents. Other points in this table will be referred to later. Since the amount of an extract need not necessarily correspond to its toxicity, the following preliminary tests were performed.

Experience has taught that the honeybee (*Apis mellifica* L.) is extremely sensitive to stomach poisons; therefore this insect was fed small quantities of the foregoing extracts in order to determine the degree of toxicity of each one. It was furthermore considered desirable to know the effect of heat on the extracts. Consequently five of these extracts were obtained without the application of heat and the other five with the use of it. The following method of procedure was employed: Since all of these extracts, except those obtained with water, have a consistency similar to that of thick paste and are not soluble in water, it was necessary to dissolve a small quantity of each in alcohol; therefore 0.4 gm. of the petroleum-ether extract was dissolved in 10 cc. of 95 per cent alcohol. The same method was employed for each one of the other nine extracts, including the water extract, so that the effect of the alcohol would be the same in all the tests; and then ¼ cc. of one of these solutions was mixed thoroughly with 5 cc. of honey in a small

feeder, which was so covered with wire that the bees could not waste any of the food. After the 10 feeders, containing supposedly poisoned food, had been placed in as many wire-screen cases, 50 normal bees were introduced into each case; the bees were thereafter observed carefully and the dead ones were counted at regular periods. As a control, honey containing the same amount of alcohol as mixed with the other food was used; and whenever the bees required more food, pure honey was given to them. These experiments were repeated and were so arranged that the probable errors were minimized. Reference to Table II (extracts No. 246–254) shows that all of these extracts, except the water extract, are almost equally toxic to the honeybee within 48 hours and that there is practically no difference in toxicity between the extracts obtained with the use of heat and without it. The water extract apparently had no effect on the bees tested. Similar results were obtained by using the same extracts against aphids, fall webworms (*Hyphantria cunea* Dru.), and tussock-moth caterpillars (*Hemerocampa leucostigma* S. and A.) (see No. 246–249, 252–253, Table IV, and No. 253, Table V). The water extract from the powder of *Derris* sp. (filtered mixtures) killed only a small percentage of the aphids sprayed (see lower half of Table IV), while the nonfiltered spray mixtures, consisting of powder and soap solution, were efficient against aphids.

To determine whether the solvents had removed all of the toxic principle from the powders extracted, these five powders (No. 240–244 in Table II) after having been thoroughly dried were fed to other honeybees in the same manner as already described. In these tests ⅛ gm. of powder was thoroughly mixed with 5 cc. of honey. Reference to Table II shows that the powders exhausted with ether, chloroform, and alcohol had very little effect on the bees tested, while the powder exhausted with water killed 94 per cent of the bees within 48 hours. The results pertaining to the powder exhausted with petroleum ether are not reliable (see note at bottom of Table II).

To ascertain the effect of powder exhausted successively with 1 to 4 of the solvents and also the effects of the resulting extracts, other experiments were performed. Reference to Table II (No. 260, 261, 264, and 266) shows that powder successively extracted is only slightly less effective than powder extracted once, and that the third and fourth successive extracts (No. 263 and 265) have no effect at all. These results agree in only certain respects with the successive quantitative extractions, expressed in Table I.

To determine whether any poisonous volatile substance can be removed from Derris by steam distillation, 50 gm. of the powder were so treated and the distillate was collected. Later some of this distillate and a portion of the distilled powder, after it had been dried, were tested on silkworms. The distillate had no effect whatever, but the powder was as poisonous as ever.

To facilitate the handling of a product which might be used as a proprietary insecticide, an alcoholic extract was incorporated into a soft linseed-oil soap at the rate of 1 gm. of extract to 4 gm of soap. This product was later dissolved in water in the proportion of 1 to 2,400, which would be equivalent to about 1 pound of powder to 200 gallons of soap solution. All of the small fall webworms sprayed with this solution died, but none of the controls sprayed with soap solution of the same strength died.

TABLE II.—*Effects on the honeybee of eating extracts and powders of Derris sp. (probably D. elliptica)*

No. of extract or powder used.	Extracts, powders, and controls.	Number of bees tested.	Percentage of bees dead within—		
			48 hours.	48 hours.	7 days.
			After eating extracts and controls.	After eating powders from which one or more extracts had been removed, and controls.	
246	Petroleum-ether extract (no heat used)	150	97	.....	.....
247	Petroleum-ether extract (heat used)	150	97	.....	.....
248	Ether extract (no heat used)	150	97	.....	.....
249	Ether extract (heat used)	150	96	.....	.....
250	Chloroform extract (no heat used)	150	93	.....	.....
251	Chloroform extract (heat used)	150	95	.....	.....
252	Alcoholic extract (no heat used)	150	96	.....	.....
253	Alcoholic extract (heat used)	150	95	.....	.....
254	Water extract (heat used)	150	5	.....	.....
	Control, alcohol in honey	150	8	.....	.....
	Control, honey alone	150	3	.....	.....
240	Powder exhausted with petroleum ether	100	.....	[a] 100	.....
241	Powder exhausted with ether	100	.....	4	.....
242	Powder exhausted with chloroform	100	.....	2	.....
243	Powder exhausted with alcohol	100	.....	6	.....
244	Powder exhausted with water	100	.....	94	.....
245	Powder not exhausted with any solvent	100	.....	98	.....
	Control, wheat flour in honey	100	.....	1	.....
258	Powder exhausted with petroleum ether	100	.....	[a] 98	[a] 100
260	Powder exhausted with above solvent and ether	100	.....	0	73
261	Powder exhausted with above solvents and chloroform	100	.....	1	16
264	Powder exhausted with above solvents and alcohol	100	.....	2	18
266	Powder exhausted with above solvents and water	100	.....	0	16
	Control, wheat flour in honey	100	.....	1	15
	Control, honey alone	100	.....	4	11
259	Petroleum-ether extract from original powder	100	67	.....	.....
262	Ether extract from above powder (2d extraction)	100	73	.....	.....
263	Alcoholic extract from above powder (3d extraction)	100	1	.....	.....
265	Water extract from above powder (4th extraction)	100	1	.....	.....
	Control, alcohol in honey	100	1	.....	.....

[a] This powder emitted an odor resembling that from petroleum ether; the bees ate very little of the honey containing it, and therefore most of them probably died for lack of suitable food.

Tables I and II show that 95 per cent ethyl alcohol is the only good economic solvent used and that heat has no effect on the extract obtained. It was decided, therefore, to make quantitative extractions of several species of Derris by using hot denatured alcohol, since this solvent is comparatively cheap.

TABLE III.—*Quantitative extractions of various species of Derris made with hot denatured alcohol*

Name of species.	Part of plant used.	Percentage of extract obtained.
Derris sp. (probably *D. elliptica*)	Roots	14.25
D. elliptica, called "tuba"	Roots	8.50
D. uliginosa	Stems	8.50
D. koolgibberah	Stems	10.30
D. scandens	Roots	20.30
D. oligosperma	Stems	22.50
D. robusta	Roots	16.70
D. robusta	Stems	15.70

The foregoing table shows that denatured alcohol is a good solvent and that the percentages of extract obtained vary considerably; this variation is certainly due in part to the fact that the eight powders used varied considerably in fineness. Results showing the effectiveness of these extracts are discussed on page 188 and in Table V.

EXTRACTION OF TOXIC PRINCIPLE FROM DERRIS SP. BY TWO METHODS

As already stated, Greshoff (*3*), van Sillevoldt (*9*), and Powers (*8*) have agreed that the toxic principle in *Derris elliptica* and. *D uliginosa* is a resin and have called the active portion of it "derrid." In the present investigation it was considered expedient to isolate a small quantity of the resin and to test it on insects and on a few higher animals.

VAN SILLEVOLDT'S METHOD

One kilo of the powdered root of *Derris* sp. was repeatedly extracted with boiling water until the extract was only slightly colored. After the powder had been filtered and thoroughly dried it was boiled under a reflux condenser with successive portions of 95 per cent alcohol until exhausted. The combined alcoholic extracts were mixed with one-fourth their volume. of water, and the alcohol was distilled under reduced pressure. As the alcohol was removed, the material in the flask became milky in appearance and the resinous substance collected in a mass on the bottom of the flask The last portion of the water was removed by transferring the material to an open dish on a steam bath. The residue was a resinous, sticky mass which weighed 110 gm., representing 11 per cent of the dry root. It was

dissolved in boiling alcohol, and then the solution was heated with animal charcoal and filtered. Upon evaporation, the resin closely resembled the appearance it had before being treated with the charcoal and seemed to consist of two forms, the greater portion being of a soft and pliable nature, while the other portion was hard and brittle. The latter had a melting point of 66°–68° C. Van Sillevoldt reports the melting point of "derrid" as being about 73° C.

Two gm. of the soft portion were dissolved in 50 cc. of 95 per cent alcohol by means of a low heat; upon standing, a fine, yellowish-white powder settled to the bottom of the flask; then this powder was separated by means of a force filter, and after being washed with a small quantity of alcohol and ether it was dried. This material appeared like an amorphous powder, but under the microscope it was found to consist of small plate-like crystals. The melting point of these crystals was 170° C.

A dilute alcoholic solution of the above crystals, as well as the alcoholic solution of the resin from which the crystals had been separated, was found to be very toxic to fish. A subcutaneous injection of 0.00066 gm. of the crystals was fatal to a mouse in two hours.

The preceding method of extracting the resin is not very practicable on a large scale. Several of the operations involved could possibly be dispensed with.

POWER'S METHOD

One kilo of the powdered root of *Derris* sp. was repeatedly extracted with boiling alcohol until exhausted. Upon removal of the alcohol by distillation under reduced pressure, 173 gm. of a dark extract of a pillular consistency were obtained; this amount is equivalent to 17.3 per cent of the dry root. Then the extract was repeatedly extracted under a reflux condenser with hot petroleum ether until the latter was no longer colored. From the combined extracts the petroleum ether was removed and a waxy, yellow residue weighing 16 gm. remained. This residue was designated *A*.

The alcoholic extract after having been exhausted with petroleum ether was heated on a steam bath with 95 per cent alcohol until it was brought into solution, whereupon it was poured slowly into a large quantity of cold water; a fine suspended precipitate resulted. The precipitated resin was filtered by means of a force filter, then washed with water, dried by means of an electric fan, and finally pulverized to a No. 20 powder which was grayish in color and weighed 102 gm., being equivalent to 10.2 per cent of the original material. This was designated *B*.

Sixty-five gm. of the resin *B* were extracted in a Soxhlet extractor with chloroform until exhausted; 11.4 gm. or 17.5 per cent remained undissolved. This portion was removed from the extractor, was dissolved in alcohol, and then precipitated in cold water. After the precipitate had

been filtered and dried, a chocolate-brown powder resulted. This was designated *C*.

The chloroform extract from *B* was placed on a steam bath to remove the chloroform. The residue resulting was a dark, sticky material which became hard and brittle when cooled below room temperature. It was ground while hard and was designated *D*.

The three substances designated *A*, *C*, and *D* were tested in very dilute form on small chinook salmon and were found to be exceedingly toxic. The extract *A* appeared to be the most powerful, while the chloroform-soluble resin *D* was much more toxic than was *C*. The effect of the extract *A* on the fish might have been influenced to a considerable extent by a trace of petroleum ether which seemed to remain in the extract and imparted to it a distinct odor.

The three substances called *A*, *C*, and *D* were tested also on small tent caterpillars by being sprayed on foliage. Within eight days *A* had killed 70 per cent, *C* 92.3 per cent, and *D* 54.4 per cent of the caterpillars tested; but only 22.1 per cent of the control larvæ had died.

EXTRACTION OF DERRIS ELLIPTICA AND TESTS OF EXTRACTS OBTAINED

The roots of "tuba" or "toeba" were ground as fine as their fibrous nature would permit, and 200 gm. of this powder were macerated for two days with a quantity of cold water. After the mixture had been filtered, the water extract measured 600 cc., each cc. representing $\frac{1}{3}$ gm. of the roots. Half of this cold water extract was tested on small tent caterpillars; within eight days only 30.9 per cent of them had died. The other half of this extract was evaporated to one-half its volume on a steam bath and then again made up to its original volume with water. This portion of the extract was later tested on small tent caterpillars; within eight days only 14.3 per cent of them had died. This does not mean that the application of heat affected the toxicity of the extract, for 22.1 per cent of the control larvæ died.

The marc from the preceding water extractions was dried by means of a current of air and was macerated with several portions of cold petroleum ether. The combined extracts were then divided into two equal portions. While the petroleum ether evaporated spontaneously from one portion in an open dish, it evaporated on a steam bath from the other portion. The residue resulting was a waxy, yellow substance which represented 1.4 per cent of the original material. Spray solutions containing these petroleum-ether extracts were tested on aphids; there was practically no difference in effectiveness between the extract obtained without the use of heat and the one with it (see No. 288 and 289, Table IV).

The powder left after the preceding extractions was spread out, and the residual petroleum ether was allowed to evaporate. It was then

divided into two equal parts; one part was macerated with successive portions of cold 95 per cent alcohol until exhausted, and the other part was boiled on a steam bath with successive portions of 95 per cent alcohol until exhausted. The combined extracts from the first part represented 4.17 per cent of the original powder and those from the second part 4.26 per cent. Spray solutions containing these alcoholic extracts were tested on aphids, small fall webworms, and on large tussock-moth caterpillars. There was practically no difference in their effectiveness on these insects (see No. 290 and 291, Table IV).

EXTRACTION OF DERRIS ULIGINOSA AND TESTS OF EXTRACTS OBTAINED

The stems were reduced to a coarse powder, and 100 gm. of this material were repeatedly extracted on a steam bath with petroleum ether until exhausted. Upon evaporation of the petroleum ether, there remained a yellow, shiny, somewhat brittle substance which represented 1.02 per cent of the original stems.

The marc from the above extraction was dried thoroughly and then exhausted with 95 per cent alcohol on a steam bath. The residue left upon the evaporation of the alcohol represented 7.82 per cent of the stems. The above petroleum-ether and alcoholic extracts were found very effective against aphids (see No. 293 and 294, Table IV).

EXTRACTION OF VARIOUS SPECIES OF DERRIS WITH DENATURED ALCOHOL AND TESTS OF EXTRACTS OBTAINED

Since the preceding results have shown that alcohol is the most suitable solvent for the toxic resins, the use of denatured alcohol as the best economic solvent was at once suggested. By the use of suitable apparatus this solvent can be recovered with very little loss and consequently can be used repeatedly.

For the tests described below, 50 gm. of powdered material in each instance were extracted with denatured alcohol on a steam bath, and the extract was concentrated to 25 cc. so that 1 cc. was equivalent to 2 gm. of material.

In the tests performed in the laboratory, the general plan for each test was to spray or dust about 500 aphids or 100 caterpillers on foliage, and then to place this foliage in a bottle of water inside a battery jar which was covered with cheesecloth. A record of the dead insects was taken at regular periods. The tests with aphids usually covered a period of 24 hours, and those with caterpillars and potato beetles (*Leptinotarsa decemlineata* Say) 10 or 12 days. The results of most of these tests are given in Tables IV and V. Table V gives chiefly the results obtained by using denatured alcoholic extracts and the powders of various species of Derris, applied as dust. Attention is called to the

following points: (1) The alcoholic extracts of *elliptica*, *uliginosa*, and *koolgibberah* (No. 296, 295, and 298) were generally efficient, while those of *oligosperma*, *scandens*, and *robusta* (No. 299, 300, 400, and 401) were only seldom efficient; (2) the powder of *Derris* sp. (No. 110), mixed with water or soap solution, was usually efficient, while the other powders (No. 402–406) tested by this method were found inefficient; and (3) of the eight powders used as dusts, only those of *Derris* sp., *elliptica*, and *uliginosa* (No. 110, 408, and 407) were found efficient.

TABLE IV.—*Effects of Derris extracts as contact insecticides and stomach poisons, comparing the results of the extracts derived with the aid of heat and without heat, and those dissolved in water and in soap solution*

Species of Derris used.	Kind of extracts and controls.	No. of the extracts used.	Temperature conditions during the extraction.	Percentage of aphids and caterpillars killed, ratio of extract to solvent, and kind and strength of solvents.										
				Myzus persicae Sulz.		*Macrosiphum liriodendri* Mon.					Fall webworm.		Tussock-moth caterpillar.	
				$a_{1:100}$	$b_{1:100}$	$a_{1:100}$	$c_{1:100}$	$d_{1:200}$	$e_{1:200}$	$f_{1:200}$	$g_{2:100}$	$h_{2:100}$	$g_{2:100}$	$h_{2:100}$
Derris sp.	Petroleum-ether extract	246	No heat used	19.6	53.2	.....	83.3	.....	.....	.....	.....	.....	.....	.....
		247	Heat used	27.0	59.1	.....	93.1	.....	.....	.....	.....	.....	.....	.....
	Ether extract	248	No heat used	64.8	92.5	.....	98.3	.....	.....	.....	.....	.....	.....	.....
		249	Heat used	54.6	90.9	.....	95.3	.....	.....	.....	.....	.....	.....	.....
	Alcoholic extract	252	No heat used	33.3	93.5	.....	95.4	73.4	92.1	100.0	65.0	44.0	66.6	60.0
		253	Heat used	62.2	75.7	.....	93.7	51.7	96.4	98.8	75.3	40.0	67.3	42.2
D. elliptica	Alcoholic extract	[i] 290	No heat used	.....	.....	72.8	98.7	86.4	99.4	100.0	81.7	73.1	60.0	88.2
		[i] 291	Heat used	.....	.....	73.1	97.3	70.0	96.5	100.0	77.7	64.4	60.0	76.1
	Petroleum-ether extract	[j] 288	No heat used	.....	.....	53.7	86.2	.....	.....	.....	.....	.....	.....	.....
		[j] 289	Heat used	.....	.....	68.8	87.7	.....	.....	.....	.....	.....	.....	.....
D. uliginosa	Petroleum-ether extract	[i] 293	Heat used	.....	.....	.....	.....	48.1	81.9	95.5	.....	.....	.....	.....
	Alcoholic extract	[i] 294	Heat used	.....	.....	.....	.....	38.2	96.1	97.4	.....	.....	.....	.....
Controls	Alcohol in soap solution	.....	.....	.....	11.6	.....	24.2	11.1	14.5	90.1	.....	15.2	.....	54.0
	Soap solution only	.....	.....	.....	.....	.....	15.9	7.0	16.7	88.2	.....	22.2	.....	25.0
	Water only	.....	.....	.....	.....	4.7	.....	9.2	4.0	1.9	31.1	.....	.....	.....
D. sp.	Soaked in water five days (filtered)	.....	.....	.....	.....	.....	57.5	.....	.....	.....	.....	.....	.....	.....
	Soaked in water five days (not filtered)	.....	.....	.....	.....	.....	98.6	.....	.....	.....	.....	[k] 35.3	.....	.....
	Soaked in water two days (filtered)	.....	.....	.....	.....	[l] 31.4	.....	.....	.....	.....	.....	.....	.....	.....
	Soaked in water two days (not filtered)	.....	.....	.....	.....	.....	.....	.....	[m] 100.0	.....	52.0	.....	.....	.....
	Soaked in soap solution two days (filtered)	.....	.....	.....	.....	.....	46.0	.....	.....	.....	.....	.....	.....	.....
	Soaked in soap solution two days (not filtered)	.....	.....	.....	.....	.....	96.5	.....	.....	.....	.....	.....	.....	.....
	Proprietary extract 1:400[k]	.....	.....	.....	.....	.....	.....	90.9	100.0	87.4	.....	.....	.....	.....

[a] Ratio of extract from 1 pound of powder to 100 gallons of water.
[b] Ratio of extract from 1 pound of powder to 100 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[c] Ratio of extract from 1 pound of powder to 100 gallons of soap solution. Soap solution made by adding 1 pound of fish-oil soap to 100 gallons of water.
[d] Ratio of extract from 1 pound of powder to 200 gallons of soap solution. Soap solution made by adding 1 pound of fish-oil soap to 100 gallons of water.
[e] Ratio of extract from 1 pound of powder to 200 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[f] Ratio of extract from 1 pound powder to 200 gallons of soap solution. Soap solution made by adding 4 pounds of fish-oil soap to 100 gallons of water.
[g] Ratio of extract from 2 pounds of powder to 100 gallons of water.
[h] Ratio of extract from 2 pounds of powder to 100 gallons of soap solution. Soap solution made by adding 1 pound of fish-oil soap to 100 gallons of water.
[i] See p. 187.
[j] See p. 186.
[k] Ratio of extract to water 1:100, soap being added later at rate of 4:100.
[l] Ratio of extract to water 2:100.
[m] Ratio of extract to water 2:100, soap being added later.

TABLE V.—*Effects of alcoholic extracts and powders of various species of Derris as contact insecticides and stomach poisons, obtained inside and outside the laboratory*

Extracts and powders used.	Species of Derris used.	Kind of extracts and powders used and controls.	No. of the extracts and powders used.	Percentage of aphids and potato beetles killed, ratio of extract to solvent, and kind and strength of solvent.										
				Illinoia sp.		*Macrosiphum liriodendri* Mon.	*Aphis helianthi* Mon.	*Rhopalosiphum pseudobrassicae* Davis.		*Aphis gossypii* Glover.	*Aphis spiraecola* Patch.	Colorado potato beetles.		
												Outside laboratory.		In potato patch.
				Inside laboratory.	Outside laboratory.	Inside laboratory.	Inside laboratory.	In turnip patch.	Inside laboratory.	Inside laboratory.	Outside laboratory.	Larvæ.	Adults.	Larvæ.
				[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[b] 1:100	[c] 1:50	[c] 1:50	[c] 1:50
	Derris sp.	Alcoholic extract	253	.....	.....	96.4	78.7	.....	.....	90.0	.....	.....	.....	.....
	D. elliptica	Alcoholic extract	[d] 291	.....	.....	96.5	86.1	.....	.....	96.0	.....	.....	.....	.....
		Denatured alcoholic extract	296	.....	.....	.....	94.8	98.0	100.0	100.0	30.0	96.6	29.1	.....
	D. uliginosa	Denatured alcoholic extract	295	.....	.....	.....	92.0	95.0	100.0	100.0	30.0	98.5	25.0	.....
		Alcoholic extract	[e] 294	.....	.....	96.1	.....	.....	.....	.....	.....	.....	.....	.....
	D. koolgibberah	Denatured alcoholic extract	298	.....	.....	.....	94.6	.....	100.0	100.0	30.0	.....	.....	.....
	D. oligosperma	do	299	.....	.....	.....	54.0	.....	60.0	65.0	20.0	.....	.....	.....
	D. scandens (roots)	do	300	.....	.....	.....	78.0	.....	95.0	82.0	30.0	.....	.....	.....
	D. robusta (roots)	do	400	.....	.....	.....	23.7	.....	99.0	50.0	30.0	.....	.....	.....
	D. robusta (bark)	do	401	.....	.....	.....	47.4	.....	99.0	60.0	30.0	.....	.....	.....
	Control	Soap solution only	.....	23.3	15.0	14.5	14.2	.....	20.0	25.0	20.0	.....	.....	.....

[a] Ratio of extract from 1 pound of powder to 200 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[b] Ratio of extract from 1 pound of powder to 100 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[c] Ratio of extract from 1 pound of powder to 50 gallons of water.
[d] Ratio of extract from 2 pounds of powder to 100 gallons of soap solution. Soap solution made by adding 1 pound of fish-oil soap to 100 gallons of water.
[e] Ratio of extract to water 1:100, soap being added later at rate of 4:100.

TABLE V.—*Effects of alcoholic extracts and powders of various species of Derris as contact insecticides and stomach poisons, obtained inside and outside the laboratory*—Continued.

Extracts and powders used.	Species of Derris used.	Kind of extracts and powders used and controls.	No. of the extracts and powders used.	Percentage of aphids and potato beetles killed, ratio of extract to solvent, and kind and strength of solvent.										
				Illinoia sp.		*Macrosiphum liriodendri* Mon.	*Aphis helianthi* Mon.	*Rhopalosiphum pseudobrassicae* Davis.		*Aphis gossypii* Glover.	*Aphis spiraecola* Patch.	Colorado potato beetle.		
				Inside laboratory.	Outside laboratory.	Inside laboratory.	Inside laboratory.	In turnip patch.	Inside laboratory.	Inside laboratory.	Outside laboratory.	Outside laboratory.		In potato patch.
												Larvæ.	Adults.	Larvæ.
				[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[b] 1:100	[c] 1:50	[c] 1:50	[c] 1:50
Extracts and powders in solutions, sprayed upon insects and plants.	*D.* sp. (probably *D. elliptica*).	Powder soaked in water ½ hour.	110	.....	.....	.....	.....	.....	.....	.....	.....	100.0	70.0	100.0
		Powder soaked in soap solution ½ hour.	110	100.0	90.0	.....	96.6	.....	.....	100.0	.....	.....	.....	.....
		Powder soaked in soap solution 2 days.	110	100.0	.....	.....	.....	.....	.....	.....	50.0	.....	.....	.....
	D. koolgibberah (stems)	Powder soaked in soap solution ½ hour.	402	75.8	50.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. oligosperma (stems)	do	403	66.7	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. scandens (roots)	do	404	60.0	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. robusta (roots)	do	405	90.6	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. robusta (bark)	do	406	70.3	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Powders dusted upon insects and plants.	*D.* sp. (roots)	Dusted	110	97.7	.....	92.3	88.6	.....	100.0	99.0	.....	.....	.....	.....
	D. koolgibberah (stems)	do	402	75.9	.....	.....	10.0	.....	95.6	50.0	.....	.....	.....	.....
	D. oligosperma (stems)	do	403	34.9	.....	.....	9.7	.....	87.5	10.0	.....	.....	.....	.....
	D. scandens (roots)	do	404	34.1	.....	.....	4.0	.....	90.6	4.5	.....	.....	.....	.....
	D. robusta (roots)	do	405	33.3	.....	.....	3.0	.....	91.0	5.0	.....	.....	.....	.....
	D. robusta (stems)	do	406	13.2	.....	.....	16.8	.....	98.3	5.0	.....	.....	.....	.....
	D. uliginosa (stems)	do	407	.....	.....	.....	.....	.....	96.0	.....	.....	.....	.....	.....
	D. elliptica (roots)	do	408	.....	.....	.....	.....	.....	100.0	.....	.....	.....	.....	.....
	Control	Wheat flour	.....	2.5	.....	.....	4.1	.....	5.8	2.0	.....	.....	.....	.....

[a] Ratio of extract from 1 pound of powder to 200 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[b] Ratio of extract from 1 pound of powder to 100 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[c] Ratio of extract from 1 pound of powder to 50 gallons of soap solution.

EFFICIENCY OF DERRIS AS AN INSECTICIDE

The experiments relating to the efficiency of Derris as an insecticide were performed at the Insecticide Board's Testing Laboratory, located at Vienna, Va. The Derris material used was purchased on the open market and is distinguished from the other materials employed by being called powder derived from *Derris* sp.

EFFICIENCY OF DERRIS AS A CONTACT INSECTICIDE

The commercial powder, when used as a contact insecticide, was applied in two forms: (a) as a dry powder and (b) as a spray mixture with or without soap.

DERRIS APPLIED AS A POWDER AGAINST VARIOUS INSECTS

DOG FLEAS.—Eight dogs badly infested with fleas (*Ctenocephalus canis* Curt.) were dusted thoroughly. The material was applied with a shaker and well rubbed into the hair with the hands. At the end of 48 hours no living fleas were observed. Several dead ones were seen still clinging to the hairs.

CHICKEN LICE.—Twelve hens badly infested with several species of lice (Mallophaga) were thoroughly treated with the powder, which was well rubbed in through the feathers. When the hens were examined two or three days later, they were free from lice.

CHICKEN MITES.—When this powder was freely dusted over the chicken mites (*Dermanyssus gallinae* Redi), confined in jars, all were killed within 24 hours, but when used under practical conditions in a badly infested chicken house, all of the mites were not killed.

BEDBUGS.—Derris was tested against bedbugs (*Cimex lectularius* L.) by placing 20 bugs in a jar with a quantity of excelsior and then thoroughly dusting the contents of the jar. In nine tests under these very severe conditions 24.4 per cent of the bugs were killed in 24 hours and 52.8 per cent in four days. This material would be of no practical value against bedbugs.

ROACHES.—Six small cages were thoroughly dusted and 20 roaches (*Blattella germanica* L.) were placed in each cage. At the end of one week an average of 57.5 per cent of the roaches were dead, which indicates that this material would be of very little value under practical conditions.

HOUSE FLIES.—In cage tests, where house flies (*Musca domestica* L.) were dusted in ordinary flytraps about 10 inches high, all were dead or inactive within 24 hours. In room tests, where the powder was freely blown into the air and all parts of the room with a small hand dust gun, all of the flies were dead at the end of 16 hours.

In one test several hundred flies were liberated in a room which had been thoroughly dusted seven days before. Twenty-four hours later very few active flies were to be seen, and on the second day only three or four were living.

PLANT INSECTS.—Derris applied as a dust was of no value against the mealy bug (*Pseudococcus citri* Risso), the Orthezia (*Orthezia insignis* Doug.), red spiders (*Tetranychus bimaculatus* Harv.), and the crawling young of the oyster-shell scale (*Lepidosaphes ulmi* L.); but it was effective against nasturtium aphids (*Aphis rumicis* L.) and the green apple aphis (*Aphis pomi* De Geer).

DERRIS APPLIED AS A SPRAY MIXTURE

Derris applied as a spray mixture was tested against the green apple aphis (*Aphis pomi* De Geer) under field conditions and was found to be very effective. Young apple trees, about 10 feet high, were used. The spray mixtures were applied with a knapsack sprayer, except in tests No. 3 and 6, in which a barrel sprayer was used. When soap was used it was employed at the rate of 1 pound to 25 gallons of water. One dusting experiment was performed, the powder being applied with a large hand duster.

TABLE VI.—*Results of field tests, using Derris powder in spray mixtures and as a dust against the green apple aphi (Aphis pomi De Geer)*

No. of test.	Ratio of powder to water or soap solution used, dusting test, and controls.	Number of trees used.	Duration of tests.	Aphids killed.
			Days.	*Per cent.*
1	1 pound of powder to 25 gallons of water	5	3	100
2	1 pound of powder to 50 gallons of water	5	3	100
3	.....do	32	4	95–100
4	1 pound of powder to 50 gallons of soap solution	2	3	100
5	1 pound of powder to 100 gallons of water	3	3	100
6	.....do	52	4	80–90
7	1 pound of powder to 100 gallons of soap solution	5	3	100
8	1 pound of powder to 150 gallons of water	5	3	100
9	1 pound of powder to 150 gallons of soap solution	5	3	100
10	1 pound of powder to 200 gallons of water	5	4	[a] 98–100
11	1 pound of powder to 200 gallons of soap solution	5	3	[a] 98–100
12	Dusting test	2	2	100
13	Control, soap solution only	4	4	0
14	Control, trees untreated	8	4	0

[a] Four trees entirely free of aphids.

Table VI shows that Derris, even at the rate of 1 pound to 200 gallons of water, was very effective against the green apple aphis under field conditions and that on apple foliage the addition of soap does not increase its effectiveness. It also shows that this powder is effective as a dust.

Under greenhouse conditions, in tests against the nasturtium aphis, this material was found to be effective when used at the rate of 1 pound

of powder to 400 gallons of water, with soap at the rate of 1 pound to 100 gallons.

OYSTER-SHELL SCALE.—At the rate of 1 pound of powder to 20 gallons of water, either with or without soap in the proportion of 1 pound to 100 gallons, Derris was ineffective against the crawling young of the oyster-shell scale (*Lepidosaphes ulmi* L.).

While taking records of numerous greenhouse tests with Derris against aphids, it was noticed that all the aphids were not killed during the first 24 hours but continued to die for several days. Since a contact insecticide which continued to kill for a period of five or six days seemed an anomaly, the following experiments were made to determine definitely if this were the case and over how long a period this killing would extend.

The aphids on small potted plants, were counted, and the plants were then thoroughly dusted or sprayed. Paper disks were placed around the plants to catch the aphids that fell. Careful counts were made every day until all of the aphids were gone. In these counts each aphid was observed through a lens, and when necessary each one was touched with the point of a knife to determine whether it was still alive. A single untreated plant was used with each series as a control.

The aphids began falling from the plants within an hour, but for the first 24 hours most of those on the paper disks were alive. After this the aphids that fell were practically all dead. In the case of the dusted plants a few dead aphids were found clinging to the leaves the third day, but as a rule only the living ones remained on the plants.

These tests fully confirmed the earlier observations and, furthermore, showed that some of the aphids did not die until five or six days after the application of the insecticide. The results are presented in Tables VII and VIII.

TABLE VII.—*Results of tests against nasturtium aphids (Aphis rumicis L.), using Derris powder in spray mixtures at the rate of 1 pound of powder to 100 gallons of water*

Number.	Percentage of aphids living on plant at end of—						
	First day.	Second day.	Third day.	Fifth day.	Sixth day.	Seventh day.	Eighth day.
Aphids treated:							
182	52.2	24.7	10.4	4.4	2.2	0.5	0.5
150	25.3	22.6	7.3	2.0	2.0	.0	.0
209	19.1	11.0	9.0	8.1	5.7	3.3	2.8
172	33.7	20.3	23.2	19.7	19.7	18.6	12.7
Average	35.0	19.6	12.7	8.5	7.4	5.6	4.0
Aphids untreated:							
159	96.2	105.6	104.4	137.1	144.6	169.7	235.2

TABLE VIII.—*Results of tests against aphids (Myzus persicae Sulz.) on cabbage plants, using Derris powder as a dust*

Number.	Percentage of aphids living on plant at end of—								
	First day.	Second day.	Third day.	Fourth day.	Sixth day.	Eighth day.	Tenth day.	Thirteenth day.	Fifteenth day.
Aphids treated:									
96	43.7	36.4	31.2	16.6	3.1	0.0	0.0	0.0	0.0
157	38.4	21.0	6.3	2.5	.6	.0	.0	.0	.0
111	49.5	29.7	11.7	9.0	4.5	1.8	.9	.9	.0
235	47.6	31.0	16.5	15.3	9.7	2.1	1.2	.4	.0
Average	44.8	29.5	16.4	10.8	4.5	1.0	0.5	0.3	0.0
Aphids untreated:									
180	103.3	128.3	146.1	179.4	231.6	315.5	315.5+	315.5+	315.5+

Reference to Tables VII and VIII shows that the percentage of untreated aphids gradually increased from the first day of the tests onward; This increase was due to the birth of aphids on the untreated plants. aphids were born likewise on the treated plants from the time the insecticide was applied until all the reproducing females had died. Since practically all of the aphids on the treated plants were dead at the close of the tests, the newly born young ones must have been killed by coming in close proximity to the particles of powder still remaining on the plants.

EFFICIENCY OF DERRIS AS A STOMACH POISON AGAINST VARIOUS INSECTS

POTATO-BEETLE LARVÆ.—Derris powder as a stomach poison was tested on a small scale against potato-beetle larvæ (*Leptinotarsa decemlineata* Say) at several strengths, ranging from 1 pound of powder to 16 gallons of water up to 1 pound to 128 gallons and was found to be very effective. Practically all of the larvæ were killed within 48 hours and the plants were little eaten.

Since these spray mixtures might have acted as contact poisons, because the larvæ were already on the plants when the latter were sprayed, a second series of tests was arranged to eliminate this factor. The same plants were used and from 20 to 40 larvæ were placed on them one or two days after they had been sprayed. The results obtained were practically the same as in the first series of tests. Very few living larvæ were found three days later and the plants were little eaten.

When applied as a dust, Derris was equally efficient against potato-beetle larvæ.

TENT CATERPILLARS.—Derris was tested against young tent caterpillars (*Malacosoma americana* Fab.) in a series of strengths ranging from 1 pound of powder to 8 gallons of water to 1 pound to 200 gallons. All the mixtures were found to be effective.

Apple tree branches were thoroughly sprayed, and after the foliage had dried from 20 to 40 newly hatched larvæ were placed on each branch. The caterpillars began to show signs of discomfort within 48 hours and were practically all dead in from 5 to 10 days. In no case was any material amount of feeding observed.

In a second series of tests the larvæ were placed on the branches and sprayed after they had begun to form their tents. Under these conditions sprays containing 1 pound of powder to 50 gallons of water and 1 pound to 100 gallons killed all of the larvæ within 24 hours. When 1 pound to 200 gallons and 1 pound to 400 gallons were used all the larvæ were not killed within 11 days, but the few which remained alive were very small and inactive.

Used as a dust, this material killed all of the treated larvæ within one week.

FALL WEBWORMS.—These caterpillars (*Hyphantria cunea* Dru.), about one-third grown, were killed within a week by a spray containing 1 pound of powder to 5 gallons of water. Mixtures ranging from 1 pound to 50 gallons to 1 pound to 200 gallons were not satisfactorily effective, since nearly all of the sprayed foliage was eaten and not all of the caterpillars were killed.

OAK WORMS.—Two small oak trees, on which about 300 caterpillars (*Anisota senatoria* S. and A.) were feeding, were sprayed thoroughly with Derris at the rate of 1 pound of powder to 25 gallons of water; soap was added at the rate of 1 pound to 50 gallons, and a knapsack sprayer was used. Within 24 hours the larvæ became inactive and ceased to feed, and at the end of 6 days no living ones could be found. As a check on this test, powdered arsenate of lead was applied at the rate of 1 pound to 50 gallons of water, and almost identical results were obtained.

A second test was made in which a small tree was sprayed, and 24 hours later about 50 larvæ were placed on it. The caterpillars ate very little and gradually disappeared, evidently leaving the tree, since no dead ones were observed; and at the end of 5 days they were nearly all gone.

DATANA LARVÆ.—Two apple trees, on which large colonies of nearly full grown apple datanas (*Datana ministra* Dru.) were feeding, were sprayed with Derris at the rate of 1 pound of powder to 50 gallons of water. Twenty-four hours later one living larva was found on one tree and two on the other. The ground under the trees was thickly sprinkled with dead larvæ and many had lodged in the trees.

CABBAGE WORMS.—In two cage tests against cabbage loopers (*Autographa brassicae* Riley), Derris, applied at the rate of 1 pound to 25 gallons of water, killed all of the larvæ within 24 hours.

PHARMACOLOGICAL EFFECTS OF TOXIC PRINCIPLE

The preceding experiments show that the toxic principle contained in Derris kills insects both as a contact insecticide and as a stomach poison. It now remains to be shown how this poison kills insects. This phase of the work involves a careful study of the physiological effects of the toxic principle on insects and of how it reaches the internal tissues.

PHYSIOLOGICAL EFFECTS

In the foregoing experiments it was observed that the various spray mixtures and powders were effective only when they came in actual contact with the insects tested. The following experiments were performed to determine whether they would kill insects without coming in actual contact with them. In these experiments only the powder from *Derris* sp. was used.

Ten small fall webworms, confined in an observation wire-screen case, were placed ⅛ inch above the surface of a strong mixture of Derris powder and water so that the exhalation and vapors from the mixture could pass freely through the wire screen. No effects on the insects were observed which could be attributed to the presence of the insecticide.

Fall webworms, ants (*Monomorium pharaonis* L.), various species of aphids, roaches, and the larvæ of *Prodenia ornithogalli* Guenée were confined in large, air-tight glass tubes with Derris powder so that they could not touch it. As a rule, the exhalation from the powder had little effect upon the confined insects. None of the webworms or larvæ of Prodenia died, and only a small percentage of the ants and aphids and only the recently hatched roaches succumbed.

Most of the aphids dusted with Derris powder fell within a few hours in a paralyzed condition from the plants bearing them, and then they lay more or less helpless for a few hours before they died. Aphids sprayed with Derris mixtures and extracts behaved almost normally and showed no symptoms of paralysis; in short, they died very slowly and their behavior was similar to that of those sprayed with quassia extracts, described by McIndoo and Sievers (*7, p. 523*). Honeybees fed extracts of Derris seemed to die of motor paralysis; and their behavior was similar to that of those fed nicotine, described by McIndoo (*6, p. 91*); but it was somewhat different from the behavior of those fed arsenic.

HISTOLOGICAL METHODS OF TRACING DERRIS POWDER AND SPRAY MIXTURES IN INSECTS

Small individuals of fall webworms, caterpillars of Datana, silkworms, and cockroaches, confined in wire-screen observation cases, were dusted with Derris powder (No. 200). Three hours later all of them were "stupid," and after being removed from the cases they were put in vials containing thick celloidin. After remaining in the celloidin an hour they were put in other vials containing chloroform. Then an hour later they were cut into

small pieces and were fixed in a liquid containing equal parts of absolute alcohol and chloroform with corrosive sublimate to excess. The thick celloidin completely covered the integuments of the dusted insects and held the particles of powder where they were already adhering to the hairs and integuments. It did not pass into the mouth, anus, or spiracles but ran into all of the crevices and surrounded the hairs. The chloroform soon made the celloidin hard, thereby forming a hard layer around the insect, and thus holding the powder in position. Sections made from this material were stained with eosin in equal parts of absolute alcohol and chloroform. This method kept the celloidin hard and thus firmly held the particles of powder in position.

A study of the sections described above showed the following: A thick layer of celloidin, dotted with particles of powder, completely surrounded the integument, and processes from it ran into all of the crevices or indentations of the integument. The heat in the paraffin bath caused the celloidin to shrink, thereby drawing it away from the integument at places; but at other places it remained in contact with the integument. Most of the powder in the layer of celloidin lay against the integument and none could be seen inside the insect, except particles here and there which seemed to have been dragged inside by the microtome knife; none was seen in the tracheæ and only occasionally was a small amount observed in the spiracles, but never enough to clog them.

To be able to trace the powder better and distinguish it from the particles of food in the intestine, the following experiments were performed: Eight fall webworms were dusted with a mixture of Derris powder and lamp-black, and eight more with a mixture of Derris powder and carmine; the lamp-black and carmine were finely pulverized and were mixed thoroughly and in equal proportions with the Derris powder. The first four of each set were three hours later fixed intact in the modified Carnoy's fluid (equal parts of absolute alcohol, chloroform, glacial acetic acid, and corrosive sublimate to excess); and the second four of each set were treated by the celloidin process, described above. Many sections were made from the material of each set; one-half of those from the material dusted with the Derris powder and lamp-black mixture were stained in the mixture of absolute alcohol, chloroform, and eosin; and the other half were left in the paraffin-ribbon stage on the slides and not stained. The sections from the material dusted with the Derris powder and carmine mixture were likewise treated, one-half being stained with methylin blue in 95 per cent alcohol and the other half being left unstained in the paraffin-ribbon stage.

A study of these sections showed the following: The black and red powders were easily traced around the outside of the integuments but never in the tracheæ, and only occasionally did a small amount lie in the mouth of a spiracle. In many of the sections, small masses of the

colored particles lay inside the integument; but most of them, if not all, seemed to have been dragged there by the microtome knife, or washed there by the staining liquid and xylol. However, a careful study of the paraffin-ribbon sections, from the material dusted with the Derris powder and carmine mixture, showed red powder only on the outside of the integument and none inside, except a small amount here and there in the intestine.

McIndoo (*6, p. 103*) has shown tnat nicotine spray solutions not containing soap do not pass into the tracheæ of certain aphids and caterpillars, and the same is true for quassia-spray solutions not containing soap. Quassia-spray solutions containing soap, however, pass freely into the tracheæ and finally reach the various tissues (*7, p. 525*). In view of these results it was not considered necessary to trace Derris extracts contained in water and in soap solution.

The preceding histological study seems to show the following: Derris powder dusted upon insects does not pass into the tracheæ, but a limited amount of it may lodge in the spiracles, though never sufficiently to interfere with breathing. In order that the vapors and exhalation from a nicotine-spray solution be effective, it is necessary for the insects sprayed to carry some of this solution on their bodies; likewise it is necessary for the insects dusted with Derris powder to carry some of this powder on their bodies in order that its exhalation may pass into the spiracles in as undiluted a condition as possible. After being dusted the insects seem to swallow some of the powder, which later may act as a stomach poison. Soap solutions containing Derris extracts pass freely into the spiracles and finally reach the various tissues, but probably the extracts kill by first affecting the nerve tissue.

SUMMARY

Derris, known widely as a powerful East Indies fish poison, was found to fulfill several of the requirements of a general insecticide; it acts both as a contact insecticide and as a stomach poison, but is of no practical value as a fumigant. Six species of Derris were tested, but only two of them (*elliptica* and *uliginosa*) were found to be satisfactory for insecticidal purposes.

According to the views of various authors, the toxic principle in Derris is a resin, which affects the various classes of animals according to the development of their nervous systems. It kills some insects easily and others with difficulty, but it usually acts slowly and seems to kill by motor paralysis.

Denatured alcohol was found to be a good economic solvent for extracting the toxic principle, which when applied in spray mixtures proved to be efficient against certain aphids, potato-beetle larvæ, and small fall webworms. For proprietary insecticides it is possible to incorporate the extracts from Derris into soft soaps which when greatly diluted with water are ready for use.

Derris powder, used as a dust under practical conditions, was found to be efficient against dog fleas, chicken lice, house flies, three species of aphids (*Aphis rumicis* L., *Aphis pomi* De Geer, and *Myzus persicae* Sulz.), potato-beetle larvæ, and small fall webworms, but of no practical value against bedbugs, roaches, chicken mites, mealybugs, *Orthezia insignis*, red spiders, or against the crawling young of the oyster-shell scale. Used as powder in water with or without soap under practical conditions, it proved to be efficient against most of the aphids sprayed and also against cabbage worms (*Autographa brassicae* Riley), the larvæ of apple datanas (*Datana ministra* Dru.), oak worms (*Anisota senatoria* S. and A.), small tent caterpillars, and potato-beetle larvæ.

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EFFECTS OF HEAT ON TRICHINÆ

By B. H. RANSOM, *Chief of the Zoological Division*, and BENJAMIN SCHWARTZ, *Junior Zoologist, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

It is a well-known fact that the larvæ of *Trichinella spiralis*, which are of rather common occurrence in pork, may be killed by thorough cooking and the meat thereby rendered safe for food so far as concerns the danger of trichinosis. As to the actual temperature required to kill the parasites, however, various writers give very different figures, so that the question of the thermal death point has been rather uncertain.

The thermal death point of trichinæ is a matter of great practical importance in connection with the control of cooking processes employed by meat-packing establishments in the preparation of cooked products containing pork. The simple rule of cooking pork until it is well done, which can be applied satisfactorily by a careful cook in the household kitchen, is not suited to conditions in meat-packing establishments. Instead of such a rule a more exact statement of requirements is desirable. In fact, the Bureau of Animal Industry, which is charged with the enforcement of the federal meat-inspection law, requires that pork or products containing pork cooked in establishments operating under Federal inspection shall be heated sufficiently to insure a temperature throughout all portions of the meat that will destroy the vitality of any trichinæ which may be present, specifically a temperature of 137° F. (58° + C.). This temperature is several degrees higher than the temperature that has been accepted by the bureau as representing the thermal death point of encysted trichinæ, but the difference between the two represents no more than a reasonable allowance as a margin of safety.

Before a decision could be reached as to the degree of heat required to destroy the vitality of encysted trichinæ, it was found necessary to supplement the investigations on this question which are recorded in the literature with further experimental work; and it is the purpose of this paper to set forth the results obtained. This work was begun by the senior writer in 1913, continued in 1914 and 1915, and in the latter part of 1915 taken up by the junior writer.

REVIEW OF LITERATURE

Haubner, Küchenmeister, and Leisering (*5*) [1] state that trichinæ are killed by prolonged salting, followed by 24 hours of smoking, but do not give data as to the temperature of smoking.

[1] Reference is made by number (italic) to "Literature cited," pp. 220–221.

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Fiedler (*1, p. 26-29*) found that if small particles of trichinous meat were heated to 35° R. (43.75° C.) in water the heating had no other effect than to render the parasites more active when viewed at the same temperature under the microscope. Similar results were obtained by heating to a temperature of 40° R. (50° C.). The trichinæ in finely chopped meat held at a temperature of 50° R. (62.5° C.) for 15 minutes and then cooled were found to show movement when gently warmed, but reexamination of the meat 24 hours later failed to show any trichinæ that would move when warmed. This experiment was frequently repeated with similar results, and similar results were obtained with a temperature of 52° R. (65° C.). Temperatures of 58° R. (72.5° C.) and upward, allowed to act for a period of 10 minutes in all cases, affected the parasites so that no movement occurred afterward when gentle heat was applied. Three rabbits and a cat were fed trichinous meat after it had been heated 10 minutes at a temperature of 50° R. (62.5° C.), and none became infected. Trichinous meat heated 10 minutes at a temperature of 40° to 42° R. (50° to 52.5° C.) infected a rabbit. In another experiment meat heated at 40° R. (50° C.) for 10 minutes failed to infect a young cat. Trichinous meat heated at 60° R. (75° C.) for 10 minutes failed to infect two rabbits.

In another paper Fiedler (*2, p. 467-468*) reported an experiment in which he fed two rabbits with minced trichinous meat that had been heated in water for 10 minutes at a temperature of 50° R. (62.5° to 65° C.). No infection resulted. He also reported an experiment in which two rabbits were fed with trichinous meat that had been heated in water for 10 minutes at a temperature of 45° to 46° R. (56.25° to 57.5° C.). No infection resulted.

Haubner (*4*) states that the smoking of pork at a temperature which reaches and exceeds 52° R. (65° C.) kills the trichinæ or brings about their early death.

Rodet (*12*) states that trichinæ do not die at a temperature of 55° to 60° C. He also asserts that they survive even a temperature of 70° to 80° C. and succumb with certainty only to a temperature of 100° C. In support of his views Rodet presents very imperfect experimental evidence. He states that he placed pieces of trichinous muscle in water at a temperature of 70° to 80° C. and allowed them to remain there for some time. Upon being taken out of the water the trichinæ in the meat were still lively. When plunged into water at 100° C. they were killed and became completely uncoiled.

Fjord and Krabbe (*3*) concluded that encysted trichinæ die at 52.5° C. after a 30 minutes' exposure. At 54° C. they survived 10 minutes and at 55° to 56° C. they died in 5 minutes. Their method of procedure consisted in cutting up trichinous meat and heating it in a vessel containing warm water while agitating the contents with a thermometer. To

determine the effects of the heating upon the vitality of the parasites they fed the meat to rabbits, which were examined for trichinæ 15 to 30 days after feeding.

Perroncito (7) records observations on the behavior of the larvæ under the influence of high temperatures and draws the conclusion that a temperature of 48° to 50° C. is sufficient to kill the parasites. He placed decapsuled larvæ as well as encysted larvæ in salt solution and examined them on a warm stage. He observed that as the temperature increased the larvæ became more active, but that at 45° C. their activities ceased. If the temperature was lowered they resumed their activities. If the temperature was raised to 48° or 50° C. they became completely inactive and remained so even when the temperature was lowered.

Vallin (*13*) records a series of experiments on the effects of heat on trichinæ. He heated small pieces of trichinous meat in tubes containing water, placed the tubes on a sand bath, and read the temperatures on a thermometer with which each tube was provided. He found that a 20-minutes' exposure to a temperature of 60° C. resulted in a complete destruction of the vitality of the larvæ. He fed the heated meat to two rabbits and four guinea pigs and failed to infect them. Vallin states that temperatures below 60° C. are uncertain in their effects, since after heating meat to 56° C. he succeeded in infecting with it one guinea pig, although two rabbits to which the meat was fed escaped infection. He tried temperatures lower than 56° C. and found them ineffective.

Leuckart (*6*) states that *Trichinella spiralis* does not perish until it is acted on by a temperature ranging between 62° and 69° C.

Piana (*8*) concluded as a result of certain experiments that a temperature of 56° C. is fatal to the larvæ of *Trichinella spiralis*.

Ransom (*10, p. 159*) states:

> With reference to the effects of high temperatures upon the vitality of trichinæ, various statements are found in the literature which seem to have for the most part rather imperfect experimental evidence as a basis. From a rather small series of experiments conducted within the last two years, I have found that encysted trichinæ regularly die when exposed for a short time to a temperature somewhere between 53° and 55° C.

The earlier of these experiments supplied the data upon which was based the following statement (*9*): "The results already obtained in the investigations . . . show that the parasites die after a brief exposure to a temperature between 53° and 55° C."

Winn (*14*) records a series of experiments in which trichinous meat was heated to certain temperatures, maintained at those temperatures for 15 minutes, and then fed to experimental animals. The effect of the heat was judged by the degree of infection as compared with that of animals fed on similar quantities of meat which were unheated. Winn found that temperatures below 53° C. produce no apparent effect upon

the vitality of the worms. At 53° C. he found the vitality of the worms slightly reduced, but the results were variable. At 54° C. there was a further reduction in vitality, but meat which was heated to 55° C. and maintained at that temperature for 15 minutes was not capable of producing an infection.

EXPERIMENTAL WORK

Experiments by the present writers on the effects of heat on the larvæ of *Trichinella spiralis* have been made with meat containing encysted larvæ as well as with larvæ freed from their capsules by artificial digestion. In the former case there is more or less difficulty in obtaining accurate data, since the temperature in the interior of the meat does not necessarily correspond to the temperature of the medium in which it is heated. This difficulty may be overcome, however, if small pieces of muscle tissue are used and if the temperature is raised gradually. In experiments on larvæ freed from their cysts by artificial digestion more accurate determinations can be made, since the temperature of the medium is an excellent index to the temperature of the parasites themselves. From a comparison of the results obtained by the two methods definite conclusions regarding the thermal death point of the larvæ may be drawn.

OBSERVATIONS ON THE SURVIVAL OF DECAPSULED LARVÆ IN VARIOUS MEDIA

In connection with experiments on the effects of heat upon decapsuled larvæ, the question of their survival in various media following artificial digestion is important, since such experiments are complicated by the factor of abnormal environment, and results obtained might not correspond with those obtained in experiments in which the parasites are subjected to heat while still inclosed in their capsules in pieces of meat. Encysted trichinæ may be kept alive for many months and may still be viable in meat that has become badly decomposed. Although decapsuled larvæ are unlikely to survive as long as encysted larvæ, they can be kept alive for considerable periods of time. In a paper by the senior writer (Ransom, *11*), it has been shown that decapsuled larvæ may retain their normal activity and appearance when kept in tap water or 0.6 per cent salt solution at a temperature of about 20° C. for a period of from 10 days to two weeks or more, and that they have been kept alive and very active for as long as 11 days in 2 per cent salt solution. On the other hand, at a temperature of 38° decapsuled larvæ kept in tap water became inactive within a few hours, whereas when kept in 0.6 per cent salt solution at the same temperature for the same length of time they suffered no apparent injury.

Further observations have been made by the junior writer which show quite clearly that the longevity of the larvæ after artificial digestion depends upon both the medium in which they are kept and the temperature

to which they are subjected. Pure water as compared to physiological salt solutions was found to be distinctly injurious, the injurious action varying directly with the temperature. Larvæ kept in distilled water at a temperature of 39°–40° C. were all dead at the end of 22 hours, while in 0.7 per cent solution of sodium chlorid or in Ringer's solution they lived longer, although they all died within 48 hours. In distilled water at a temperature of 32°–33° decapsuled larvæ were nearly all uncoiled at the end of 48 hours, while in 0.7 per cent sodium-chlorid solution or in Ringer's solution some were still alive at the end of 5 days. Similar differences were observed in the case of lower temperatures. In distilled water at 25°–26° larvæ remained alive for 4 days; in physiological salt solutions at 25°–27° some were still alive at the end of 13 days; in distilled water kept at a temperature of about 8° only a few larvæ were still alive at the end of 12 days; while in physiological salt solutions at the same temperature some larvæ were still alive at the end of 50 days.

From these observations and our general knowledge of the phenomena of osmosis it would appear that the loss of salts from the tissues of the worms into the water and the penetration of the water into the tissues of the worms are important factors in bringing about the death of the worms when kept in hypotonic media, such as distilled water. This belief is borne out also by the fact, noted in a former paper (*11, p. 849*) and repeatedly observed since that paper was written, that larvæ kept in a hypotonic solution until they have begun to show distinct evidence of its effects, such as loosening of their coils and paling of their protoplasm, if transferred to a physiological salt solution before the injurious action of the hypotonic medium has gone too far, will usually resume a normal state of contraction and a normal or almost normal brown color. Another indication that the death of decapsuled larvæ kept in hypotonic solutions may be dependent upon osmotic processes is that they die more quickly at high than at low temperatures, which is in harmony with the fact that osmosis is hastened by raising the temperature.

Another factor or factors, however, enter into the matter, inasmuch as in isotonic solutions as well as in hypotonic solutions the larvæ do not survive so long at high temperatures as at low temperatures. It may be supposed that at the higher temperatures death of the larvæ kept in isotonic and comparatively inert solutions is brought about by exhaustion resulting from the greater activity of the worms and consequently more rapid oxidation of their tissues than at lower temperatures. Such an explanation is complicated by the fact that larval trichinæ encysted in the muscles of a living animal may live for many years, although constantly subjected to a temperature at which they live only two or three days when removed from their cysts and kept in salt solutions. Possibly in the living animal they are kept in a relatively inactive condition through the operation of factors no longer present when they are removed

from their normal environment, and it is possible also that they may be able to replace waste through the absorption of nutritive materials from their host.

A natural corollary to experiments on the effects of hypotonic solutions are experiments on the effects of hypertonic solutions. A typical example of such an experiment is one in which decapsuled larvæ were kept for 22 hours in a molar solution of dextrose. At the end of this time they were found to be partially uncoiled; their protoplasm was dull in appearance; the cuticle was wrinkled, particularly in the posterior portion of the body; the body wall was wrinkled; and the cells of the esophagus were indistinct. After having been transferred to and kept in 0.7 per cent salt solution overnight, they were found to be tightly coiled and normal in appearance. Similar results were obtained in a repetition of this experiment.

So far as concerns the purposes of the present paper, the foregoing observations are of interest because they show that trichinæ freed from their cysts by artificial digestion may be kept alive for a long time in physiological salt solutions, in water, and in certain hypertonic solutions, and that, although within a temperature range the upper limit of which does not exceed 40° C. their longevity decreases as the temperature at which they are kept is raised, they do not in any case die quickly.

EXPERIMENTS WITH DECAPSULED LARVÆ

Inasmuch as trichina larvæ that have been freed from their cysts by digestion of finely chopped trichinous meat in artificial gastric juice[1] at a temperature of 38° to 40° C. for a period of about 20 hours can be kept alive for long periods of time, they can be conveniently used in experiments on the effects of heat. In a medium such as a 0.6 per cent or 0.7 per cent solution of sodium chlorid, but also in plain water if not kept too long, they display more or less activity even at ordinary room temperatures but commonly assume a posture in which they are tightly coiled spirally; and their movements are often limited to a tightening or loosening of the coil. Their protoplasm, when unaffected by heat or other injurious agents, exhibits a certain brilliancy in appearance; and pigment in the cells of the alimentary tract, especially of the esophagus, gives them a distinct brownish color. After a little experience, departures from the normal both as to their behavior and appearance of their protoplasm can easily be detected by microscopic examination. As a rule, in experiments in heating decapsuled larvæ, the larvæ were placed in a beaker or test tube containing sometimes water but usually a phys-

[1] The following fluid has yielded satisfactory results:

Scale pepsin (U. S. P.)	2.5 gm.
Sodium chlorid	2 gm.
Hydrochloric acid (sp. g. 1.19)	10 cc.
Water	1,000 cc.

iological salt solution or Ringer's fluid; and this was heated to the desired temperature on a water bath over an open flame, or in an incubator. After being cooled, individuals were removed with a pipette to hollow ground slides, or in some cases transferred to a Petri dish or shallow stender dish and allowed to cool. They were then examined directly on a warm stage, either on slides or in the dishes, in order to determine the results of the experiment.

BEHAVIOR OF DECAPSULED LARVÆ WHEN HEATED

When trichina larvæ are heated on a warm stage their reactions may be directly observed with the microscope. As the temperature rises they begin to uncoil and become very active, their activity gradually increasing. When the temperature has reached the neighborhood of 50° C. spasmodic contractions are commonly observed, and the larvæ twist themselves into various shapes. With a further rise of temperature they grow sluggish and may become either uncoiled and inactive or else tightly coiled and quiescent. After passing into this sluggish condition they may again become lively if the temperature is lowered, but if subjected to a sufficiently high temperature for a sufficient length of time they do not recover when removed to a cool place.

Decapsuled trichinæ killed by heat usually become uncoiled and assume a characteristic shape resembling the figure 6. If allowed to stand for some time the protoplasm becomes dull, certain granulations appear, and often the cell partitions in the gonads can no longer be distinguished. Larvæ in this condition are readily recognizable as dead. Sometimes, however, larvæ that have been subjected to heat may remain loosely coiled and the protoplasm may not undergo any conspicuous changes. From experience it has been learned that larvæ in this condition are usually dead. A generally satisfactory test of life is heat stimulation; if still viable the larvæ will usually uncoil and move. Even individuals with a minimum amount of vitality will move the anterior or posterior end very sluggishly. However, the most reliable test of life, or at least of their viability from a practical standpoint, is feeding them to experimental animals and thus determining their ability to reproduce; and this has been done in some instances but not so regularly as in experiments on encysted trichinæ.

DETAILS OF EXPERIMENTS

Some experiments on the effects of heat on decapsuled trichinæ were made by the senior writer in 1913, 1914, and 1915, after which the work was taken up by the junior writer and continued along the same general lines.

EXPERIMENT 1 (April 5 and 7, 1913).—A decapsuled larva was sealed under a cover glass in salt solution on a slide and heated to 54° C. on a

warm stage. The temperature was held at 54° for a few moments. The worm was inactive at this temperature but resumed its movements when the slide was cooled. The same worm was reheated to 55° and became entirely motionless at this temperature. The temperature was raised to 55.5° and the slide then cooled. The worm became active again on cooling.

Another decapsuled larva was heated in the same manner. It became sluggish in its movements and coiled up at a temperature of 48° C. The temperature was raised slowly to 56°, and the slide was allowed to cool as soon as this temperature was attained. The worm resumed its active movements when cooled. In order to check the correctness of the temperature indicated by the thermometer in this experiment, some crystals of diphenylamin having a melting point of 54° were placed on a slide under a cover glass and heated on the stage. They melted when the thermometer registered 54°. A second trial gave the same result.

On April 7, a decapsuled larva was heated as described above. The temperature was raised slowly to 56° C. and then held for five minutes at 56° to 56.5°. When cooled the worm did not resume its movements, its internal structure showed slight disorganization, and it was undoubtedly dead.

EXPERIMENT 2 (March 28, 1914).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from three trichinous rats, were heated in a beaker of constantly stirred water over a hot water bath to a maximum of 53.6° C., 10 minutes being required for the temperature to rise to this point from 30°. The temperature dropped to 46.2° in another 10 minutes, after which 233 larvæ were examined at room temperature. All were inactive. Unheated larvæ from this lot when examined at room temperature were active. Another lot of larvæ from the same source was heated in the same manner, the temperature rising from 20° to 51° in 21 minutes, and then dropping in 6 minutes to 45.8°. One hundred and thirty-nine larvæ were then examined at room temperature, and 65 of them were found to be inactive. Of the 74 active larvæ, all but 2 were sluggish. A third lot of larvæ from the same source was heated in the same manner from 24° to 50° in 12 minutes, and then cooled to 46° in 6 minutes. Out of 159 examined, 18 were inactive. Some of the 141 active larvæ were sluggish.

EXPERIMENT 3 (May 16, 1914).—Decapsuled larvæ, isolated by artificial digestion from a mixture of meat from two trichinous rats, were heated in a beaker of constantly stirred water over a water bath. The temperature was raised from 23° to 48.4° C. in 8 minutes and held at 48.4° 1 minute. The beaker was then allowed to cool. One hundred and ten larvæ were examined on a warm stage. Thirty-five were inactive and 75 active, mostly very lively. Another lot of larvæ from the same source was heated in the same manner from 22° to 51° in 10 minutes.

Examination of 213 larvæ on a warm stage showed 179 inactive and 34 active, most of them very lively. Another lot was heated from 30° to 51.9° in 10 minutes. Ninety-nine were examined, and of these 93 were inactive and 6 active. Another lot was heated from 30° to 53° in 4 minutes. One hundred and eighteen were examined, and of these 72 were inactive and 46 active, sluggish. Another lot was heated from 22° to 53° in 12 minutes. One hundred and forty-seven were examined, and of these 109 were inactive and 38 active, sluggish. As a control upon the results of this experiment 158 unheated larvæ from the same source as those subjected to heat were examined on a warm stage. Of these 22 were inactive and 136 active.

EXPERIMENT 4 (November 17, 1914).—Decapsuled larvæ, isolated by artificial digestion from the meat of a trichinous hog, were heated in a beaker of water over a hot water bath for a period of 10 minutes, during which time the temperature gradually increased from 23° to 53.4° C. The beaker was then cooled. Seventeen of the larvæ were examined on a warm stage and one was observed to move slightly. Fifteen minutes later the larvæ remaining in the beaker were reheated to a temperature of 53.6° C., seven minutes being required to raise the temperature to this point from 38°. Twenty-four larvæ were examined after this reheating; one exhibited definite movements on a warm stage. The others were more or less tightly coiled and presumably still alive. Thirteen minutes later the larvæ remaining in the beaker were heated a third time, the temperature being raised rapidly (in 3 minutes) from 43° to 55°. Thirty-nine larvæ were examined; all were motionless and failed to react to heat, evidently dead.

EXPERIMENT 5 (November 17, 1914).—Decapsuled larvæ from the same source as those used in Experiment 4 were heated in the same manner from 16° to 54° C., 7½ minutes being required for raising the temperature. Twenty-three larvæ were examined after heating and all were found to remain inactive on a warm stage. The remainder of the larvæ in the beaker were left on the laboratory table until the following day when 42 of them were examined on a warm stage heated to 45°. Most of these were inactive but more or less tightly coiled. Thirty-five others were placed on a warm stage heated to 61°. Six of these exhibited convulsive movements before they succumbed to the heat, the others showing no response to stimulation.

EXPERIMENT 6 (November 17, 1914).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from six trichinous hogs, were heated in a beaker of water over a hot water bath to a temperature of 53.4° C. Some of them showed signs of life when examined on a warm stage. The beaker was reheated to 55°. Fifty larvæ were then examined on a warm stage and all were found to be dead.

EXPERIMENT 7 (December 19, 1914).—Decapsuled larvæ, isolated by artificial digestion from meat of a trichinous hog, were heated in 0.6 per cent salt solution in a corked bottle over a water bath. The temperature, determined by a thermometer inserted through the cork, rose from 24.4° to 56.7° C. in 44 minutes and remained at this maximum for 30 seconds, after which the bottle was allowed to cool, the temperature dropping to 34.4° in 38 minutes. Three hundred and sixty-five of the larvæ were then examined on a warm stage and all were found to be inactive. As a control on the results of this experiment 22 unheated larvæ from the same source were examined on a warm stage; 4 were inactive, 18 active.

EXPERIMENT 8 (April 6, 1915).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from six hogs, were kept 7 days in 0.6 per cent salt solution at ordinary room temperature. Some were then heated in a beaker of the salt solution, constantly stirred, over a water bath. The temperature rose from 20° to 54° C. in 7 minutes, and remained at this maximum for 30 seconds, after which the beaker was allowed to cool. Examination of some of the larvæ from the beaker showed that most of them were more or less uncoiled, but some were tightly coiled and practically normal in appearance. The beaker was kept until the following day at ordinary room temperature and the contents again examined. The great majority of the worms were still alive, but most of them were not tightly coiled.

Another lot of larvæ from the same source was heated in a similar manner but more slowly, the temperature rising from 23° to 54.8° C. in 56 minutes, remaining at 54.8° for 1 minute, after which the beaker was allowed to cool. Four hundred and seventy larvæ were examined; all were uncoiled, and their protoplasm was rather dull in appearance. The beaker was kept at room temperature until the following day, when examination of 200 larvæ showed that all were dead.

Subsequent experiments on the effects of heat on decapsuled larvæ were performed by the junior writer.

EXPERIMENT 9.—Decapsuled trichinæ in a physiological salt solution were placed in a test tube and a thermometer immersed in the solution. The test tube was placed in a beaker of water, which was heated rapidly until the thermometer registered 55° C. This temperature was attained in four minutes. The contents of the test tube were immediately transferred to a stender dish and allowed to cool. The larvæ were then examined. Nearly all were unaffected. A few days later this experiment was repeated, increasing the time of heating to about eight minutes. Similar results were obtained.

The results of other experiments with various lots of decapsuled larvæ are shown in the following table:

TABLE I.—*Effect of various temperatures on decapsuled larvæ*

Maximum temperature.	Time required to reach maximum temperature.	Results.
°C.		
53	Not recorded	Some alive.
53	50 minutes	Nearly all alive.
54	Not recorded	Some alive.
54	....do	Do.
54	42 minutes	All dead.
54.6	54 minutes	Do.
54.8	Not recorded	A few showing sluggish movements.
55	.....do	None active.
55	.....do	Do.
55	77 minutes	All dead.
55	60 minutes	All expanded.
55	65 minutes	Do.
55	37 minutes	All dead.
56	Not recorded	Do.
56	52 minutes	Do.
56	83 minutes	Do.

EXPERIMENT 10.—A 0.6 per cent salt solution was heated to 56° C. At this point some decapsuled larvæ were spurted into the solution from a capillary pipette. The temperature dropped from 56° to 55° in 75 seconds, and the contents of the vessel were then emptied into a shallow dish and examined. Of 25 larvæ, 14 were uncoiled and 11 tightly coiled. The same experiment was repeated. Of 21 larvæ, only 3 were completely uncoiled. In another test the larvæ were spurted into the solution at 55° after which the temperature was allowed to drop to 54°, which required 85 seconds. On examination following transfer to a shallow dish, only 3 out of 18 larvæ were found to be completely uncoiled.

In order to control the results of direct examination of decapsuled larvæ after heating, the junior writer in two instances fed some of the larvæ to rats. Thus larvæ heated rapidly to 55° C. in Experiment 9 were fed to two rats, which when killed at the end of a month were found to be moderately infected, a result in agreement with the results of direct examination of the larvæ. In another case—one of the experiments summarized in Table I—larvæ heated gradually for 60 minutes to 55° were fed to two rats, which were found free from trichinæ a month later. Another rat fed unheated decapsuled larvæ from the same source became infected.

From the foregoing experiments it is evident that decapsuled trichina larvæ are killed by a temperature of 55° C., provided this temperature is gradually attained. Many may be killed by lower temperatures, but the results of heating to temperatures lower than 55° are uncertain. It is also apparent that a momentary exposure to a temperature of 55° is not sufficient to destroy the vitality of decapsuled larvæ, as is shown by the results of Experiments 1, 9, and 10.

EXPERIMENTS WITH ENCYSTED LARVÆ

The experiments on decapsuled larvæ were supplemented by experiments on encysted larvæ in their natural location in pieces of infested muscle, the earlier of these experiments being made by the senior writer, the later, as noted, by the junior writer.

EXPERIMENT 11 (March 31, 1913).—Small pieces of meat from a trichinous rat were placed in a beaker of water (about 500 cc.) in a constant-temperature oven. The temperature of the water increased from an initial temperature of 18.4° to 48.4° C. in 1 hour and 10 minutes, at which time a piece of the meat was removed. Ten minutes later, when another piece was removed, the temperature had reached 51°. Eleven minutes after this at a temperature of 52.8° another piece was removed. After another period of 15 minutes, when the temperature had reached 55°, another piece was removed. Thirty-seven minutes later, when the thermometer registered 59.8°, another piece of meat was removed. A few larvæ were isolated by dissection from these various pieces of meat and examined under the microscope. The larvæ from the pieces heated to 48.4° and 51° were alive and active. One out of four larvæ from the piece heated to 52.8° showed slight movements; the others were inactive. Those from the pieces heated to 55° and 59.8° were inactive when examined. The results of direct examination of the larvæ were checked by feeding the various pieces of meat to guinea pigs. The guinea pigs fed with the meat which had been heated to 48.4° and 51° became heavily infected; those fed the pieces heated to 52.8°, 55°, and 59.8° remained free from trichinæ.

EXPERIMENT 12 (April 1, 1913).—Several small pieces of rat muscle were placed in a vessel containing 500 cc. of water and heated in an oven from an initial temperature of 16° to a temperature that reached 55° C. at the end of two hours. Pieces of meat were removed at temperatures of 51.2°, 52.2°, 53°, and 55°. A few larvæ from each piece of meat thus removed were isolated and examined directly on a warm stage. Samples from these pieces of meat were also fed to guinea pigs, which were killed about a month after feeding. The direct examination of the larvæ on a warm stage showed that, with the exception of those from the meat heated to 55°, the majority were alive and responded to thermal stimulation. Those heated to 55° were loosely coiled and did not become active on the warm stage.

The results of the feeding experiments were as follows: The guinea pig that was fed meat heated to 51.2° C. was killed seven days after feeding because it became sick. The muscles were negative, but one pregnant female trichina was found in the intestine. The guinea pig that was fed meat heated to 52.2° was killed about five weeks after feeding, and only one encysted larva was found in the diaphragm. No parasites were found in the intercostal muscles. The guinea pig that was fed meat

heated to 53° was killed five weeks after feeding and was free from parasites. The meat heated to 55° also failed to infect two guinea pigs.

EXPERIMENT 13 (April 1, 1913).—Small pieces of meat from a trichinous rat were heated as in the previous experiment; but an open flame was used instead of an oven and the temperature was allowed to go up very rapidly, the water in the beaker meanwhile being stirred constantly. Meat heated from 27.8° to 53° C. in 3½ minutes was fed to a guinea pig and resulted in a mild infection. Meat heated from 27.8° to 52° in 3 minutes and from 20° to 49.2° in 6 minutes when fed to guinea pigs produced heavy infections.

EXPERIMENT 14 (April 3 and 4, 1913).—A small piece of meat from a trichinous rat was heated in a beaker of water which was constantly stirred. The temperature rose from 17° to 53° C. in 13 minutes and remained between 53° and 53.6° for 2 minutes. One larva afterwards isolated by dissection was inactive except at the anterior end which moved slightly; another was active, though the appearance of its protoplasm was somewhat altered.

Another piece of meat was similarly heated from about 20° to 54° C. in about 10 minutes. Larvæ isolated by dissection were alive and active. Another piece was similarly heated from 28° to 53° in 11 minutes and remained in the water another minute, during which time the temperature rose to a maximum of 53.8°. Larvæ isolated by dissection were alive and active. Two pieces were heated from 28° to 55° in 13 minutes. One piece was held at a temperature of 55° for 1 minute, the other piece at the same temperature for 2 minutes. Trichinæ isolated by dissection from these pieces were inactive. Another piece of meat from the same rat was heated from 30° to 54° in 5 minutes and held at a temperature of 54° to 54.8° for 1 minute. Larvæ isolated by dissection were found to be inactive.

EXPERIMENT 15 (April 9, 1913).—Small pieces of meat from two trichinous rats were tied in a cloth around the bulb of a thermometer, which was immersed in a beaker of water and heated. The temperature was held at 54.6° to 54.8° C. for five minutes. Ten larvæ were afterwards isolated by dissection. All were inactive except one, which showed a very slight movement of its anterior end.

EXPERIMENT 16 (May 16 and 19, 1914).—Portions of the diaphragm of a trichinous rat were heated in a beaker of water stirred constantly over a water bath. Trichinæ were dissected out of the meat after heating and examined under the microscope at room temperature. A portion was heated from 24° to 54° C. in four minutes. Four larvæ examined; 1 inactive; 3 active, sluggish. Another portion was heated from 24° to 53° in 6 minutes. Ten larvæ examined; all active. Another portion was heated from 23° to 54° in 5 minutes. Twelve larvæ examined; 3 inactive; 9 active but very sluggish; appearance of protoplasm abnormal.

In the following tests portions of the diaphragm of another rat were heated. A portion was heated from 24° to 54° C. in 5½ minutes. Ten larvæ examined; 9 inactive; 1 active, very sluggish. A portion was heated from 24° to 52° in 4¾ minutes. Ten larvæ examined; all active, lively. A portion was heated from 24° to 58° in 3¼ minutes. Ten larvæ examined; all inactive. A portion was heated from 26° to 53° in 3½ minutes. Five larvæ examined; all active but not very lively. A portion was heated from 26° to 55° in 4 minutes. Twenty-three larvæ examined; 21 inactive; 2 active, very sluggish. A portion was heated from 24° to 52.6° in 9 minutes. Twelve larvæ examined; 2 inactive; 10 active, but very sluggish; appearance of protoplasm abnormal. A portion was heated from 23° to 52.9° in 2½ minutes. Eight larvæ examined; all lively. A portion was heated from 22° to 52° in 3¾ minutes. Twenty-four larvæ examined; all lively.

EXPERIMENT 17 (May 20, 1914).—Portions of the diaphragm of a third rat were heated as in Experiment 16, but more gradually. Examination was made as in Experiment 16. A portion was heated from 26° to 53° C. in 12½ minutes and cooled to 48.8° in 5 minutes. Sixteen larvæ examined; all active, but sluggish; appearance of protoplasm duller than normal. A portion was heated from 23.2° to 52° in 14 minutes and cooled to 46° in 7 minutes. Thirteen larvæ examined; all active, fairly lively but not as vigorous as unheated larvæ; no conspicuous change in appearance of protoplasm; larvæ not coiled as tightly as normal larvæ. A portion was heated from 23° to 55° in 16 minutes and cooled to 50° in 5 minutes. Fifteen larvæ examined; all inactive; protoplasm dull and dead in appearance. A portion was heated from 37° to 54° in 9 minutes and cooled to 49.4° in 6 minutes. Twenty-three larvæ examined; all active but very sluggish; protoplasm dull and dead in appearance. A portion was heated from 27° to 54° in 11¾ minutes and cooled to 49° in 5 minutes. Twenty-four larvæ examined; 16 inactive; 8 active but very sluggish; protoplasm dull and dead in appearance.

Experiments on encysted trichinæ were made by the junior writer as follows:

EXPERIMENT 18.—Small pieces of meat from a rat killed one month after infection with trichinæ were heated in a physiological salt solution to 52°, 53°, 54°, and 55° C., respectively, and then allowed to stand in a refrigerator for two days. The larvæ were then freed from their capsules by teasing out the meat, and examined directly. Those heated to 52° were still tightly coiled, although a number of loosely coiled larvæ were also seen. Most of the larvæ heated to 53° were uncoiled, but a few were coiled normally. Those heated to 54° and 55° were entirely uncoiled, dull in appearance, and failed to become active when warmed.

EXPERIMENT 19.—Larger pieces of meat from a trichinous hog were heated as in the experiment just described, kept in a refrigerator for two days, and then fed to mice. The post-mortem examinations yielded negative results in all cases.

The results obtained from the experiments in which pieces of trichinous meat were heated agree with the results of those in which the larvæ were first freed from their cysts by artificial digestion and then heated in water or physiological salt solution. The larvæ are killed if the meat is gradually heated to a temperature of 55° C., though some may escape if the temperature rises rapidly to 55° and soon falls again. They may survive a temperature of 54°; but meat which has been exposed to a temperature of about 53°, gradually attained, is likely to be non-infective.

It may be concluded that meat which has been heated so that the temperature throughout reaches 55° C. (131° F.) will be innocuous so far as concerns the possibility that persons eating such meat will become infected with trichinæ, inasmuch as under ordinary conditions of cooking the rise of temperature will be gradual enough to insure the destruction of the parasites if the temperature of the meat actually reaches 55° C. or higher. Under the regulations of the Bureau of Animal Industry the minimum temperature that must be attained throughout all portions of pieces of pork or products containing pork that are cooked in establishments operating under federal meat inspection has been fixed somewhat higher than 55° C., namely 137° F. (58.33° C.), which allows a margin of safety of several degrees above the temperature that has been shown by our investigations to be fatal to trichinæ.

THE EFFECTS UPON TRICHINÆ OF CONTINUED EXPOSURE TO HEAT AT TEMPERATURES BELOW THE THERMAL DEATH POINT

It has been shown that trichina larvæ are killed by brief exposure to a temperature of 55° C., gradually attained; and since they will not afterwards resume their activity when thus heated, this temperature may be considered the thermal death point. The vitality of the larvæ may be destroyed also by exposure to lower temperatures, provided the application of heat is long enough continued. In the former case it may be assumed that death results from irreversible coagulations of the protoplasm, in the latter case either as the result of coagulation changes which become irreversible if the heat acts for a sufficient period, or as the result of exhaustion following excessive activity to which the larvæ are stimulated by heat. We may, therefore, distinguish three ranges of lethal temperatures: The highest, in which death comes quickly from rapid and irreversible coagulations of the protoplasm; an intermediate range, in which death results probably from somewhat similar coagulation changes, changes, however, from which the parasites may more or less completely recover if the temperature is lowered before death occurs; and the lowest range, in which death is apparently brought about by exhaustion from increased activity.

The following experiments to determine the effects of the continued exposure of decapsuled larvæ to temperatures below 53° C. were carried out by the junior writer. The larvæ in 0.7 per cent salt solution or in Ringer's solution were first heated to a given temperature and then placed in an incubator at the same temperature for a given period. When taken out of the incubator the larvæ were kept at room temperature at least an hour before they were examined.

EXPERIMENT 20.—In one test the larvæ were all dead after exposure for three hours to a temperature of 48° C., but generally an exposure to a temperature of 48° for less then four hours failed to destroy their vitality. In every case, however, after they were heated four hours at a temperature of 48° they were all uncoiled, having assumed the shape of the figure 6; and they failed to react to heat stimulation.

EXPERIMENT 21.—When exposed to a temperature of 49° C. nearly one-half the larvæ in one lot were still alive at the end of two hours. Another lot from a different host animal succumbed to a similar treatment, but in no case did a briefer exposure to 49° prove effective. When subjected to 49° for 3¼ hours all the larvæ became completely uncoiled, rigid, and insensitive to thermal stimuli.

EXPERIMENT 22.—At a temperature of 50° to 50.6° C., the vitality of the larvæ was completely destroyed after an exposure of 1 hour and 20 minutes. At a constant temperature of 50° an exposure of 1½ hours proved fatal.

EXPERIMENT 23.—An exposure of one hour to a temperature of 52° C. was sufficient to destroy the vitality of decapsuled larvæ.

From the foregoing experiments it is evident that decapsuled trichina larvæ die in a comparatively short time when exposed to temperatures in the neighborhood of 50° C. and that the time required for their destruction increases as the temperature is lowered. If the results of these experiments are considered in connection with the question of the length of time that decapsuled larvæ survive at temperatures ranging below 40°, already discussed in this article, it may be concluded that between limits at which the larvæ become altogether quiescent because of the effects of heat on the one hand and of cold on the other their longevity varies inversely with the temperature. It would, however, not be safe to conclude from the experiments just described that exposure of trichina larvæ to the temperatures given for the stated periods of time would be sufficient in all cases to destroy the vitality of the parasites. It is not improbable that in these experiments the larvæ had already become somewhat exhausted as a result of abnormal activity during the process of artificial digestion, and furthermore it is possible that different lots of trichinæ vary considerably with respect to their store of vitality. The following experiments by the senior writer show that the vitality of encysted trichinæ as well as that of decapsuled

trichinæ may be destroyed by continued heating at temperatures lower than that which kills on brief exposure. Like the experiments with the decapsuled larvæ, however, they are not sufficiently extensive to allow definite conclusions to be drawn as to the periods of time necessary to insure the destruction of trichinæ exposed to temperatures lower than the thermal death point.

EXPERIMENT 24 (April 7, 1913).—A small piece of the diaphragm of the same rat which supplied the meat used in Experiment 14 was tied in a cloth around the bulb of a thermometer, which was immersed in a beaker of water heated to about 50° C. and the entire apparatus placed in a constant-temperature oven. The temperature, as indicated by the thermometer, varied from 50.2° to 51.6° during the two hours of heating the meat. Larvæ isolated from the meat by dissection were dead.

EXPERIMENT 25 (April 9, 1913).—Two small pieces of meat from the same rat used in Experiments 14 and 24 were tied in cloths around the bulbs of two thermometers and heated in a beaker of water as in Experiment 24. During the experiment the temperature, as indicated by the thermometers, varied between 49.6° and 50° C. One piece was removed after an hour's exposure. Two larvæ isolated from the meat by dissection were alive, but rather sluggish. The other piece was removed after an exposure of 1½ hours. Two larvæ were examined, one of which was dead, the other alive, but rather sluggish. Two guinea pigs were fed with the meat, but neither became infected. Another piece of meat from the same rat was similarly heated for one hour at a temperature of 50.1° to 50.4° C. A larva isolated from the meat after heating was alive and active. Another piece was similarly heated for 1½ hours at 50°. Five larvæ were isolated from the meat and examined. Four were certainly dead, the other inactive, but with protoplasm less changed than that of the others.

EXPERIMENT 26 (August 31, 1914).—Finely chopped meat from a trichinous rat was placed in water in a flask, which was kept 21 hours in an oven maintained at a temperature of 49° to 52° C. The temperature of the water during this time varied from 48.8° to 51.4°. Four larvæ dissected out of the meat after heating were dead. The meat was fed to two rats, both of which remained free from trichinæ. Some finely chopped meat from the same rat was heated 21 hours in a covered Petri dish in the same oven at a temperature of 49° to 52°. Five larvæ dissected out of the meat after heating were dead. Two rats to which the meat was fed remained free from infection.

EXPERIMENT 27 (September 3, 1914).—Finely chopped meat from a trichinous hog was heated in a closed jar in a constant-temperature oven for 19 hours. The temperature of the meat during this time varied between 47.8° and 48.4° C. Twenty-five trichinæ were dissected out of the meat after heating and all found to be dead.

EXPERIMENT 28 (September 8, 1914).—The eviscerated carcass of a trichinous rat was heated 17 hours in an oven at a temperature of 48° to 50° C. On removal from the oven the carcass had a bad odor; the upper surface was dried, the lower still moist. Twenty trichinæ were dissected out of the meat after heating and all found to be dead. Meat from the carcass was then fed to two rats, one of which remained free from trichinæ, while the other was found moderately infected when killed three months after feeding.

EXPERIMENT 29 (September 19, 1914).—Finely chopped meat from a trichinous rat was heated 5 hours in an oven at a temperature of 48° to 49° C. A few trichinæ afterward dissected out of the meat were shrunken, but their protoplasm was bright in appearance. After being soaked in water for 30 minutes some of the larvæ became lively, and 2 days later the remainder of the isolated larvæ kept in water at room temperature had also become active and normal in appearance. Some of the same meat was left in the oven until September 21, and thus exposed for 48 hours to a temperature of 48° to 49° C. It was hard and dry. Trichinæ isolated from the meat by dissection after it had been softened by soaking were very clear, pale, motionless, and apparently dead.

Additional data regarding the effects of the continued action of temperatures below the thermal death point were obtained by the junior writer. In these experiments, which are summarized in tabular form (Table II), the method of procedure was as follows: Meat from trichinous hogs was finely chopped by passing it through a meat chopper several times. A bottle with a capacity of about 200 cc. was half filled with the meat. Through a perforation in the cork a thermometer was inserted into the bottle and the top of the cork then paraffined. The bottle of meat was placed in a constant-temperature oven and the temperatures read on the thermometer in the bottle.

Inasmuch as the meat before being placed in the oven was kept in a refrigerator at a temperature of 8° to 10° C., a considerable period was required to bring its temperature near that of the oven. In nearly all the experiments shown in Table II the meat was in the oven about 2 hours before the first reading of the thermometer, given in the table as the minimum temperature, was made. Between the first and the final reading there was a slight fluctuation of the temperature but nearly always between the limits recorded in the table.

At the end of each experiment a portion of the meat was artificially digested in the usual way and the condition of the larvæ noted. As a control on the microscopic findings in each experiment two rats were fed portions of the meat, being given an average of about 10 gm. each. Unless they died earlier the test animals were killed about a month after feeding. The following table gives the record of 10 experiments:

TABLE II.—*Effects of continued action of temperatures below thermal death point on encysted trichinæ*

Temperature.		Period of exposure to given temperatures.	Appearance of larvæ after artificial digestion.	Results of feeding experiments.
Minimum.	Maximum.			
°C.	°C.	*Hours.*		
52	54	4	Coiled	Negative.
53	54.5	4½	Apparently dead	Do.
51	56	3⅙	Profoundly disorganized	Do.
52	54	4	Showing evidence of having been partially digested	Do.
50	52	4½	Uncoiled; evidently dead	Do.
49	50	5	Apparently dead	Do.
50	50	6	Probably dead	Do.
50	50	3½	Uncoiled and pale	Do.
52	53	6	.....do	Do.
50	54.8	4	.....do	Do.

From a practical standpoint the results of the experiments on the effects of continuous heating at temperatures below the thermal death point of trichinæ are of comparatively little importance so far as concerns the destruction of the vitality of trichinæ in fresh pork by cooking. Obviously, as compared to cooking at a higher temperature for a short time, there would be no advantage in subjecting meat to a lower temperature, which would require a very great lengthening of the period of heating. If for no other reason, the probable spoiling of the meat would preclude the use of such a method of destroying the vitality of the parasites. In connection with the preparation of certain kinds of cured pork products, however, the fact that heating at low temperatures for considerable periods of time is destructive to the vitality of trichinæ has been put to practical use. In this case there is also another factor which comes into play—namely, the destructive action of salt in hypertonic percentages, which increases greatly as the temperature increases. The question of the destruction of trichinæ in cured pork by heating at low temperatures will be discussed in another paper.

CONCLUSIONS

The vitality of the larvæ of *Trichinella spiralis* is quickly destroyed by exposure of the parasites to a temperature of 55° C., gradually attained, the result apparently of irreversible coagulation changes in the protoplasm. This temperature may be considered the thermal death point.

Trichina larvæ exposed to temperatures slightly below 55° C. for short periods of time may recover from this exposure; but they die if exposed for longer periods, recovery or death depending apparently upon whether or not beginning coagulation of the protoplasm has proceeded beyond a stage from which a return to normal may occur.

Exposed to temperatures in the neighborhood of 50° C., trichina larvæ die if the application of heat is sufficiently long continued, apparently as a result of exhaustion following excessive activity to which they are stimulated by the heat.

The longevity of trichina larvæ freed from their cysts by artificial digestion and kept at temperatures ranging between limits at which they become quiescent from the effects of heat and cold, respectively, varies inversely with the temperature.

Methods of destroying trichinæ by heating at temperatures below the thermal death point, which may be utilized in connection with the preparation of certain kinds of cured pork products, appear not to be applicable in the case of fresh pork.

Upon the basis of the results of experiments recorded in this paper the Bureau of Animal Industry has selected a temperature of 137° F. (58.33° C.) as the minimum temperature to which pork and products containing pork are required to be heated when cooked in establishments operating under federal meat inspection.[1] This temperature is several degrees above the thermal death point of trichina larvæ, thus providing a certain margin of safety.

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[1] This requirement has reference to the temperature actually reached in the interior of the meat and not merely to that of the water or oven in which it is cooked. It should also be understood that when meat is cooked for purposes of sterilization because of conditions other than trichinosis a higher temperature is necessary than that sufficient to destroy trichinæ.

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EFFECT OF REMOVING THE PULP FROM CAMPHOR SEED ON GERMINATION AND THE SUBSEQUENT GROWTH OF THE SEEDLINGS

By G. A. Russell

Expert, Office of Drug, Poisonous, and Oil Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Heretofore but slight attention has been paid to the germination of camphor seed. The few statements on this subject which occur in the literature refer only to the percentage of seeds germinating under conditions existing at the place of experimentation, and all the recorded results indicate a uniformly low germination. Likewise in Florida, previous to the experiments recorded in this article, the germination of camphor seed has been extremely low.

In commercial plantings in Florida, in which unpulped seeds have been planted with a modified cotton-dropping machine, the average number of seedlings brought to transplanting age on 1 acre of seed bed has been approximately 20,000. To plant an acre of seed bed requires 3 bushels of camphor seed, or approximately 200,000 seeds. The germination on a commercial scale, therefore, has averaged only about 10 per cent, which corresponds closely with the results obtained in various foreign countries. As a consequence of this low germination there has been no considerable extension of large plantings because of the limited number of seedlings available each year.

EXPERIMENTS IN 1916–17

In the fall of 1916 it was decided to make germination tests of camphor seed to determine if possible the cause or causes of the low germination obtained both experimentally and commercially. Accordingly seed was gathered from six individual trees growing in the vicinity of Orlando, Fla.

Seed from one parent tree, A, was selected from a row grown for shade and ornamental purposes. This tree was 20 years old and a typical representative of the camphor trees in Florida from which seed is gathered for commercial planting. The conditions under which the various lots of seed were collected and the treatment of each before planting are shown in Table I.

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TABLE I.—*Condition and treatment of camphor seed selected for germination tests conducted in 1916–17*

PARENT TREE A

Experimental row No.	Treatment of seed just previous to planting.
6	As they came from the parent tree.
7	Pulp removed.
10 North half	Pulp removed. Soaked in water at 25°C. for ½ hour.
10 South half	Pulp removed. Soaked in water at 50°C. for ½ hour.
13	Picked up from the ground. Pulp removed.
14	Picked up from the ground as they fell from the parent tree.
15	As they came from the parent tree. Gathered after a severe freeze.
16	Pulp removed. Gathered after a severe freeze.
17 North half	As they came from the parent tree. Gathered after a severe freeze and soaked in water for 18 hours.
17 South half	Pulp removed. Gathered after a severe freeze and soaked in water for 18 hours.

The first experiments were conducted in the winter of 1916–17. From some previous experience it was found that by removing the pulp from around the seed, germination was hastened if not materially increased. It was decided, therefore, to give special attention to the effect of removal of the pulp from the seed, since if it proved to be a decided aid to germination, the adoption of this method of treatment by commercial growers would be of distinct advantage. The remainder of the seed from the selected trees was pulped and planted. The percentage of germination was high, but the results are not recorded here since no data were secured on unpulped seed from the same trees.

The seed bed had been well prepared one week previous to the planting of the first seed, and a quantity of dry velvet-bean vines had been turned under. Drills from 1½ to 2 inches deep were opened with a hoe and the seed carefully hand-planted at intervals of 2 inches. The soil was placed back in the drill and very firmly packed. At the time of planting the soil was moist and in good condition, but later in the spring after the seedlings were several inches high it became necessary to water the bed three times in order to maintain the moisture content. On May 7 and August 1, 1917, the seed bed was fertilized with goat manure analyzing: Moisture 20 per cent, ammonia 1.5 per cent, and potash (as K_2O) 2.5 per cent. One hundred pounds were used at each application, which was at the rate of 1 ton per acre. The seedlings were well cared for by cultivating and hoeing. Table II gives the date of planting, rate of germination, and total percentage of germination in the 1916–17 trial of seed from parent tree A.

TABLE II.—*Rate and percentage of germination of camphor seed in the experiment of 1916–17*

PARENT TREE A

Experimental row No.	Date seed was gathered.	Date seed was planted.	Number of seeds planted.	Date first plants observed above ground.	Rapidity and percentage of germination.												Total germination.
					1917.										1918.[a]		
					Feb. 20.		Mar. 20.		Apr. 1.		Apr. 15.		May 10.		Jan. 4.		
	1916.			1917.	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*Per cent.*
6	Nov. 25	Dec. 1	1,000	Feb. 20	8	0.8	51	5.1	81	8.1	85	8.5	94	9.4	92	9.2	9.4
7	...do	Dec. 2	1,000	Jan. 23 [b]	171	17.1	550	55.0	556	55.6	543	54.3	522	52.2	468	46.8	60.1
10 North half	...do	...do	250	Jan. 23 [c]	32	12.8	131	52.4	131	52.4	127	50.8	121	48.4	111	44.4	60.4
10 South half	...do	...do	250	Jan. 23 [d]	14	5.6	118	47.2	118	47.2	113	45.2	104	41.6	98	39.2	61.2
	1917.			1917.													
13	Jan. 3	Jan. 6	940	Mar. 12	.....	.....	91	9.6	147	15.6	140	14.8	132	14.0	122	13.0	15.6
14	...do	...do	1,000	Mar. 20	.....	.....	11	1.1	55	5.5	59	5.9	56	5.6	51	5.1	5.9
15	Feb. 6	Feb. 10	1,000	Apr. 1	.....	.....	.....	.....	2	.2	22	2.2	45	4.5	45	4.5	4.5
16	...do	...do	1,000	Mar. 12	.....	.....	55	5.5	326	32.6	316	31.6	302	30.2	285	28.5	32.6
17 North half	...do	...do	500	Apr. 1	.....	.....	.....	.....	3	.6	8	1.6	13	2.6	17	3.4	3.4
17 South half	...do	...do	500	Mar. 9	.....	.....	56	11.2	176	35.2	196	39.2	176	35.2	164	32.8	39.2

[a] Transplanting time.
[b] Forty-five plants frozen off Feb. 2, 1917, taken into calculation in determining maximum germination.
[c] Twenty plants frozen off Feb. 2, 1917, taken into calcullation in determining maximum germination.
[d] Thirty-five plants frozen off Feb. 2, 1917, taken into calculation in determining maximum germination.

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No germination was secured when the seeds were artificially dried at a temperature of approximately 55° C. Seeds that were allowed to air-dry for several weeks in an attic likewise failed to germinate. Soaking in water at a temperature as high as 50° neither hastened nor impaired germination. Pulped seed treated with sulphuric acid of 5 per cent concentration by weight failed to germinate.

At the commercial planting beds so much seed is received in a fermenting condition that it was deemed advisable to ferment one lot of seed during an extended period in order to ascertain the effect on their germinating power. One thousand seeds fermented for 35 days in a closed jar failed to show a single case of germination. At the end of this period the pulp surrounding the seeds had almost entirely decomposed and the resulting liquid was sufficient to cover practically all the seeds. Shipments of seed for commercial use, however, are seldom enroute longer than from 8 to 10 days and do not reach such an advanced stage of decomposition. No marked ill effects due to fermentation have been noted in the commercial seed beds, which is attributed to the fact that the seed pulp has not entirely decomposed and that the liquid is constantly leaching from the barrels and boxes in which the seed is shipped, thus eliminating any chance for the seed to soak. Moreover, as soon as the seeds reach the camphor plantation they are spread out to cool and dry and fermentation ceases.

The results obtained from seed picked up from the ground are of special interest, such seed being often used in commercial work. Camphor seeds even when quite ripe do not drop readily from the tree; and a large percentage of the seeds which fall early in the season are defective, since the fallen unpulped seed showed a germination of only 5.9 per cent as compared with 9.4 per cent germination of seed picked from the tree. However, these defective seeds when pulped showed a germination of 15.6 per cent as compared with 60.1 per cent of pulped seed picked from the same tree. The seeds picked up from the ground were planted one month later than those picked from the tree, but they had fallen during the interim.

The idea that frozen camphor seed will not germinate is widely disseminated throughout Florida. A special experiment was made with seeds obtained after a relatively hard freeze during which the temperature fell to 26° F. The results obtained prove beyond doubt that camphor seed subjected to a freeze will germinate (fig. 1). This fact is of special value since freezing weather is liable to occur at any time during the late fall months in the camphor-seed producing areas, especially in those farthest north. The total germination, however, is decreased, being approximately 50 per cent of that obtained with unfrosted seeds. A greatly increased germination of the seed is secured by removing the pulp before planting. This increase was found to amount to 539 per cent. A graphic representation of the increased germination is presented in

figure 1, which shows not only the increased germination when the pulp is removed but the variation in germination of seed secured under varying conditions. The seed planted on December 1, 1916, which was picked from parent tree A, was first-class in every respect. On January 6, 1917, a quantity of seed was planted that was picked up from the ground under the same tree, and on February 6 frozen seed from this tree was secured and planted. In every instance the removal of the pulp before planting greatly increased the germination.

The percentage of germination of the seed picked up from the ground is much less than that of seed picked from the tree (fig. 1). This difference in germination was anticipated and was due in great part at least to defective seeds which fell from the tree, in other words, those which are considered as "drops." However, even the germination of these "drops" increased by 16.4 per cent when the pulp was removed. Seed picked from the tree after a severe freeze germinated remarkably well, especially when the pulp was removed. By soaking these frozen seeds in water at a temperature of approximately 25° C. for 18 hours a rather remarkable result was obtained. The

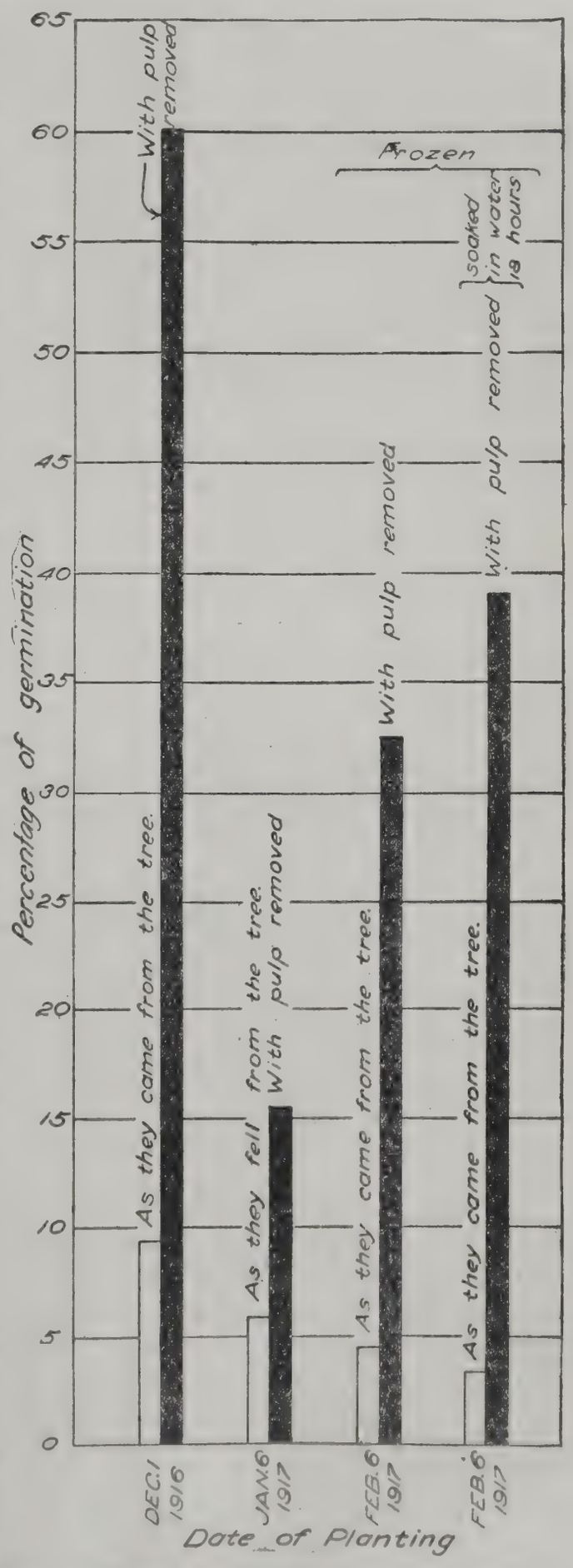


FIG. 1.—Diagram showing percentage of germination of camphor seed secured from parent tree A under varying conditions.

percentage of germination of unpulped seed was reduced, whereas that of pulped seed was increased 23 per cent over the germination of the pulped seed not soaked. The reduction in germination when the unpulped seeds were soaked can not be attributed to the direct action of the water alone. Since soaking favors a more rapid decomposition of the pulp when the seed is placed in the ground, the degree of fermentation reached may have been sufficient to destroy the vitality of some of the seeds.

In addition to the marked effect on the rapidity of germination which was noted when the pulp was removed from the seed, it was also noted that as the planting season advanced germination was more rapid and much less time was required to reach the point of maximum germination.

TABLE III.—*Rapidity of germination of camphor seed secured from parent tree A under various conditions*

Experimental row No.	Treatment of seed.	Date of planting.	Days required to reach maximum germination.
		1916.	
6	As they came from the tree	Dec. 1	161
7	Pulp removed	Dec. 2	120
10 North half	Pulp removed. Soaked in water at 25° C. for ½ hour	...do	109
10 South half	Pulp removed. Soaked in water at 50° C. for ½ hour	...do	109
		1917.	
13	Picked up from the ground. Pulp removed	Jan. 6	84
14	Picked up from the ground. As they fell from the tree	...do	98
15	As they came from the tree. Gathered after hard freeze	Feb. 10	89
16	Pulp removed. Gathered after hard freeze	...do	49
17 North half	As they came from the tree. Gathered after severe freeze and soaked in water 18 hours	...do	89
17 South half	Pulp removed. Gathered after severe freeze and soaked in water 18 hours	...do	64

Three special points of interest are brought out in Table III: First, the time for camphor seed to reach maximum germination; second, the shortening of this time by removing the pulp before planting; and third, the decrease in time required for the seed to germinate as the season advances. These points are more fully illustrated in the graph showing the time required for camphor seed to reach maximum germination (fig. 2). In every trial the pulped seed germinated much more quickly than the unpulped seed, irrespective of the condition at the time of gathering. As the season advanced and the soil warmed up, germination naturally took place in a shorter time. But what is of more interest from the commercial point of view is the fact that seed gathered and planted early in the fall will remain in the ground in good condition

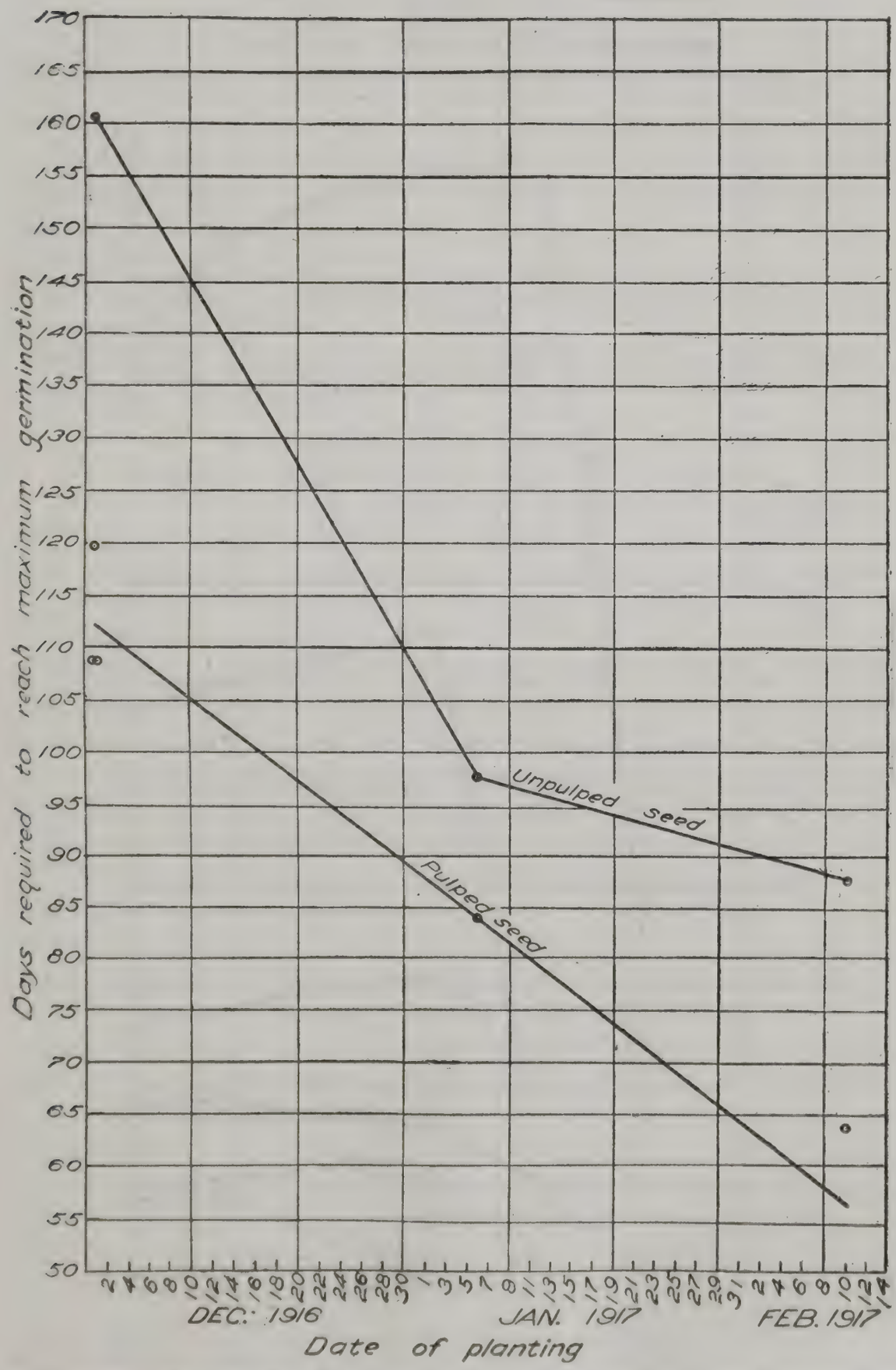


FIG. 2.—Graph showing time required for pulped and unpulped camphor seed to reach maximum germination. The seeds were planted at intervals of approximately one month during the winter of 1916–17.

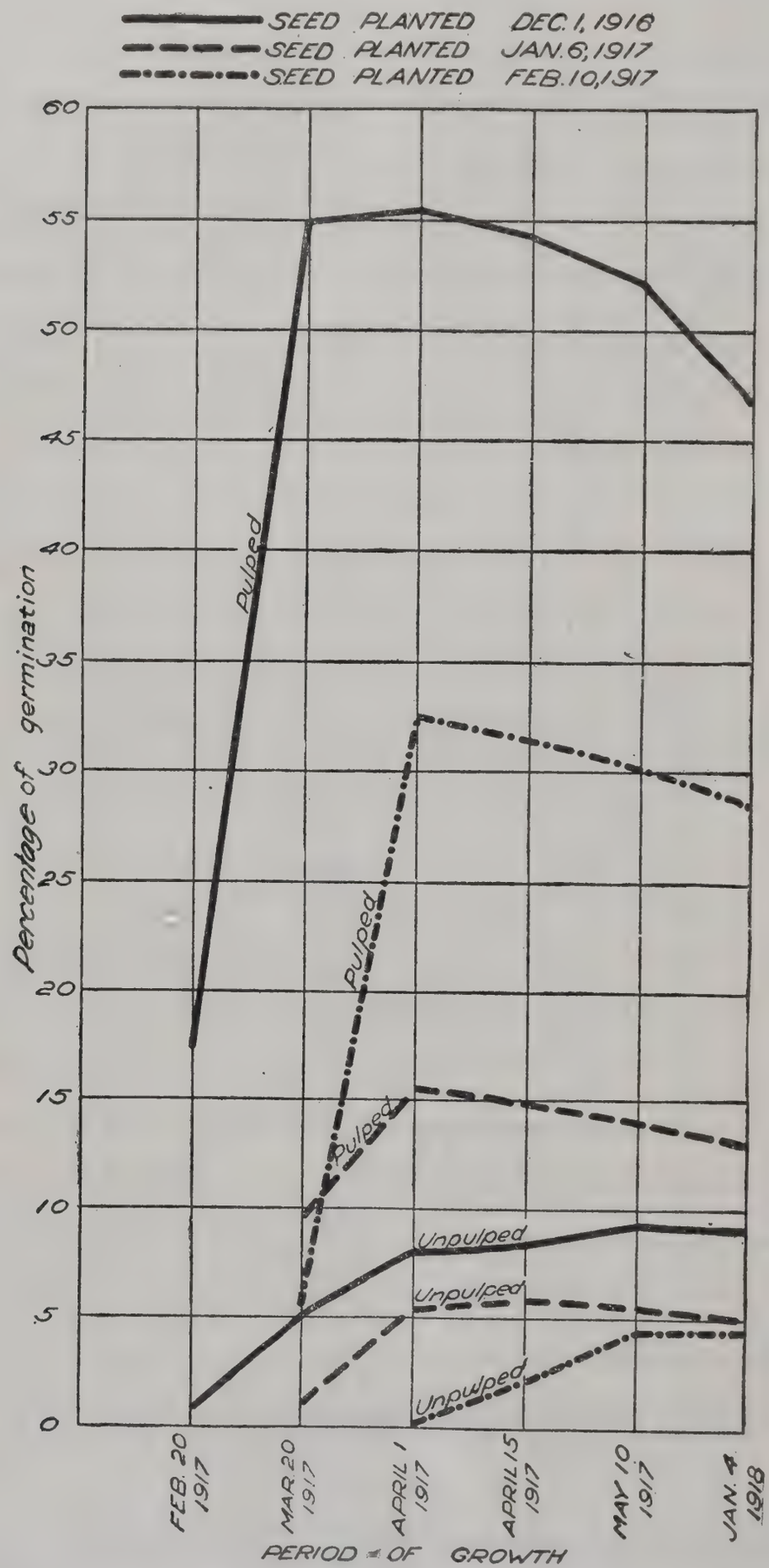


FIG. 3.—Graph showing time required for camphor seed secured from parent tree A at various times and under various conditions to reach maximum germination. The percentage of germination is also shown.

until favorable germinating weather is reached. Figure 3 shows graphically the time required to reach maximum germination and the percentage of germination of seed secured from parent tree A at various times and under various conditions. The maximum germination of all pulped seed was reached by April 1, 1917, although the time of planting extended over the period from December 2, 1916, to February 10, 1917; whereas the maximum germination of the unpulped seed occurred about May 10, 1917, the planting period extending over the same period of time as that of the pulped seed.

The results of the germination experiments of 1916–17 were so pronounced in favor of removal of the pulp from the camphor seed that the work for 1917–18 was planned to include a more extended comparison between pulped and unpulped seed.

EXPERIMENTS IN 1917–18

During the first week of November, 1917, a seed bed was prepared on soil practically identical with that employed in the experiments of 1916–17. A quantity of fertilizer made by composting rose-geranium leaves and stalks—after distilling the volatile oil—was turned under at the time the seed bed was plowed. This fertilizer material consisted only of leaves and stalks and, being somewhat intact at the time of application, had a tendency to keep the soil from packing, at the same time supplying some plant food as it decomposed. At the time of planting the soil was moist, and no subsequent watering of the bed was necessary throughout the time of the experiments. No fertilizer was applied during the growing season. The plants were given the usual cultivation and hoeing. Commercial conditions, with the exception of the application of fertilizer, were approximated as closely as possible.

Seed was selected from a row of ornamental camphor trees, and trees were chosen which bore an abundance of fruit. Camphor seed which ripens on the tree falls readily into the hand when picked. All the seeds used in these experiments were fully ripened and easily secured by picking, care being taken to secure seed from all sides of the tree. Each sample therefore was representative of the entire yield of the individual tree. All the seeds were gathered on November 27, 1917, and planted November 28, 1917. A severe freeze occurred February 2, 1918, but as none of the seedlings had appeared above ground no damage was done. In Table IV are given data in reference to the treatment of the seed, rapidity of germination, and percentage of total germination.

TABLE IV.—*Rate and percentage of germination of camphor seed in the experiments of 1917–18*

Parent tree.	No. of row.	Treatment of seeds planted.	Number of seeds planted.	Rapidity and percentage of germination. 1918. Feb. 11. No.	Feb. 11. Per ct.	Feb. 23. No.	Feb. 23. Per ct.	Mar. 18. No.	Mar. 18. Per ct.	Apr. 27. No.	Apr. 27. Per ct.	July 6. No.	July 6. Per ct.	Dec. 30. No.	Dec. 30. Per ct.	Total germination. Per ct.
A	1 North half	None	300	....	....	2	0.6	2	0.6	18	3.0	18	3.0	18	3.0	3.0
	1 South half	Pulp removed	300	48	16.0	199	66.0	223	74.0	232	77.0	232	77.0	232	77.0	77.0
B	2 North half	Pulp removed	375	137	36.5	311	82.9	325	86.6	317	84.5	317	84.5	317	84.5	86.6
	2 South half	None	500	....	....	15	3.0	67	13.4	117	23.4	101	20.2	101	20.2	23.4
C	3 North half	None	500	....	....	11	2.2	22	4.4	55	11.0	55	11.0	55	11.0	11.0
	3 South half	Pulp removed	500	49	9.8	345	69.0	375	75.0	315	63.0	259	51.8	259	51.8	75.0
D	4 North half	Pulp removed	500	181	36.2	362	72.4	367	73.4	367	73.4	377	75.4	377	75.4	75.4
	4 South half	None	500	....	....	15	3.0	40	8.0	80	16.0	69	13.8	69	13.8	16.0
E	5 North half	None	500	....	....	3	.6	9	1.8	80	16.0	63	12.6	63	12.6	16.0
	5 South half	Pulp removed	500	65	13.0	218	43.6	314	62.9	326	65.2	310	62.0	310	62.0	65.2
G	6 North half	Pulp removed	500	273	54.6	372	74.4	388	77.6	382	76.4	442	88.4	442	88.4	89.6
	6 South half	None	500	....	....	15	3.0	42	8.2	81	16.2	63	12.2	63	12.2	16.2
H	7 North half	None	500	....	....	3	.6	4	.8	35	7.0	33	6.6	33	6.6	7.0
	7 South half	Pulp removed	500	142	28.4	301	60.2	360	72.0	362	72.4	373	74.6	373	74.6	74.6
K	8 North half	Pulp removed	500	157	31.4	315	63.0	336	67.2	316	63.2	349	69.9	349	69.8	73.8
	8 South half	None	500	....	....	2	.4	11	2.2	43	8.6	31	5.2	31	5.2	8.6
O	9 North half	None	500	....	....	7	1.4	29	5.8	43	8.6	37	7.4	37	7.4	8.6
	9 South half	Pulp removed	500	114	22.8	332	66.4	397	79.4	375	75.0	382	76.4	382	76.4	80.8
P	10 North half	Pulp removed	500	151	30.2	394	78.8	409	81.8	352	70.4	418	83.6	418	83.6	95.4
	10 South half	None	500	....	....	8	1.6	31	6.2	40	8.0	36	7.2	36	7.2	8.0

In this trial the seeds from a total of 10 individual trees were tested. The results obtained by merely pulping the seed before planting were so favorable that commercial planters adopted the pulping plan when its merits were brought to their attention. In commercial work the pulp is removed by rubbing the seeds through a wire screen of the proper mesh. Many of the pulps are left behind and are swept off the screen. Those that fall through with the seed cause no inconvenience in planting, for the seed is spread to dry for about 24 to 48 hours; and during this time the pulps dry and shrink to such an extent that they readily pass through the plates of the corn planter which is now used to plant the pulped seed. Unless the seed is dried before planting the plates of the planter become clogged, causing an uneven distribution of the seeds in the row.

TOTAL GERMINATION OF CAMPHOR SEED

The greatly increased germination obtained when the seed is pulped is remarkable. Figure 4 shows graphically the total germination of both the pulped and unpulped seed from 10 parent trees. The increased germination of the pulped over the unpulped seed ranged from 270 per cent for tree B to 2,466 per cent for tree A, the average increase for the entire lot of 10 trees being approximately 525 per cent.

Germination was found to be uneven with seed from various parent trees. Moreover, the ratio between the percentage of germination of the unpulped and the pulped seed was by no means constant; and no correlation can be established between the percentage of germination

when the seed is pulped and when it is not. The variation in germination of 10 individual lots of seeds from as many parent trees is shown graphically in figure 5. The upper line in each case indicates the germination of pulped seed, the lower line the germination of unpulped seed. Under period of growth, *a* indicates the date of planting, November 28, 1917. The percentage of seeds germinating was determined by counting the number of seedlings in the beds at stated times, indicated in the figure as follows: *b*=February 11, 1918; *c*=February 23, 1918; *d*=

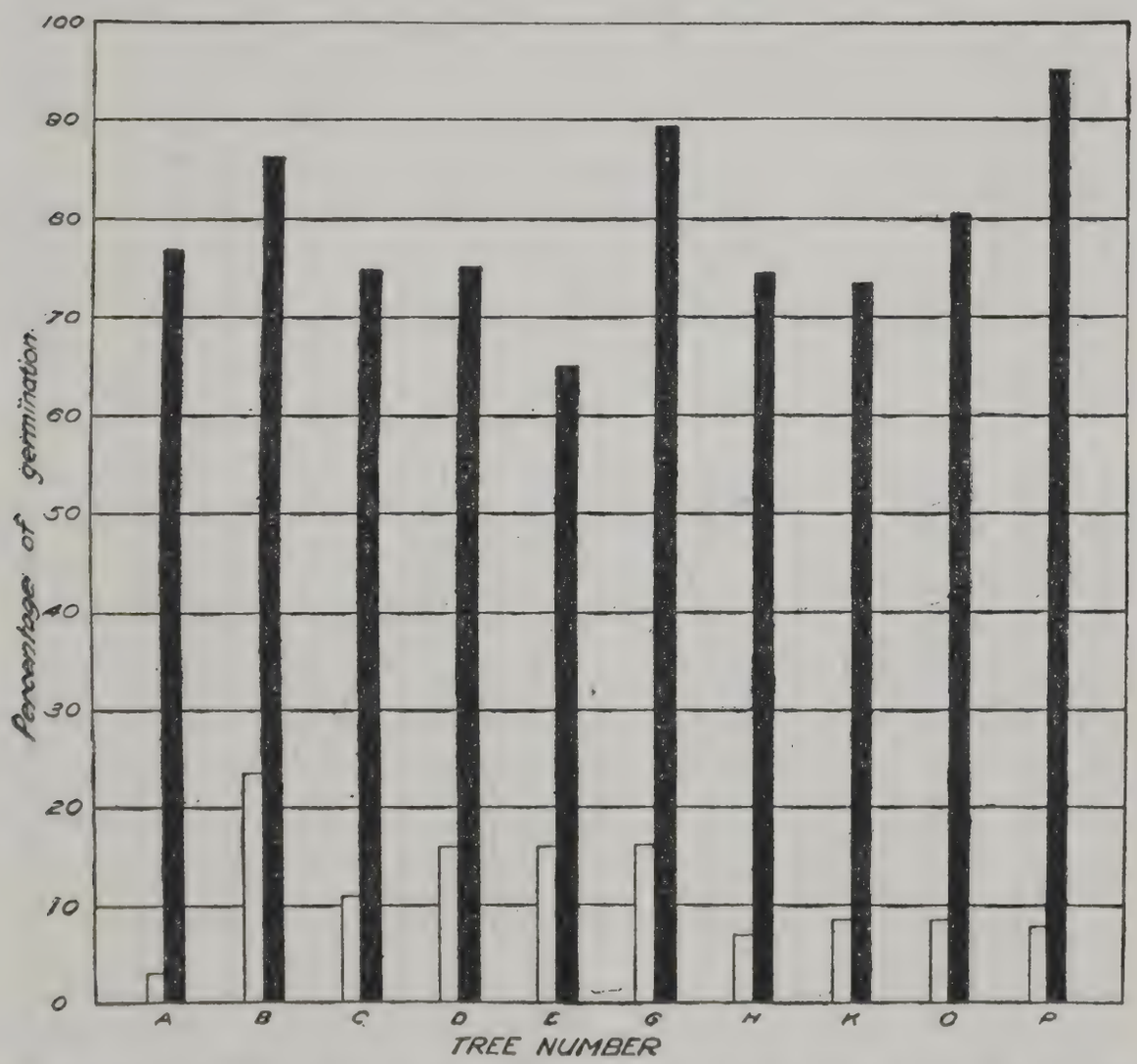


FIG. 4.—Diagram showing percentage of total germination of pulped and unpulped camphor seed from 10 parent trees. Black bars represent pulped seed; white bars, unpulped seed.

March 18, 1918; *e*=April 27, 1918; *f*=July 6, 1918; *g*=December 1, 1918, on which date the seedlings were transplanted. It will be noted that the pulped seed germinated in much shorter time than the unpulped seed and that after the appearance of the first seedlings the major portion of the germination took place in a relatively short time. The apparent falling off in the germination of the pulped seed, as indicated in the graphs (fig. 5), is due to the effect of the hot sun on the tender seedlings. A large number of the seedlings were burned off at the ground level soon after they pushed up through the hot sand, and

as a result many of those represented in the count of one day had disappeared by the time of the next count. Likewise some of the seed that germinated never entered into the calculations, the seedlings being lost to observation between counts.

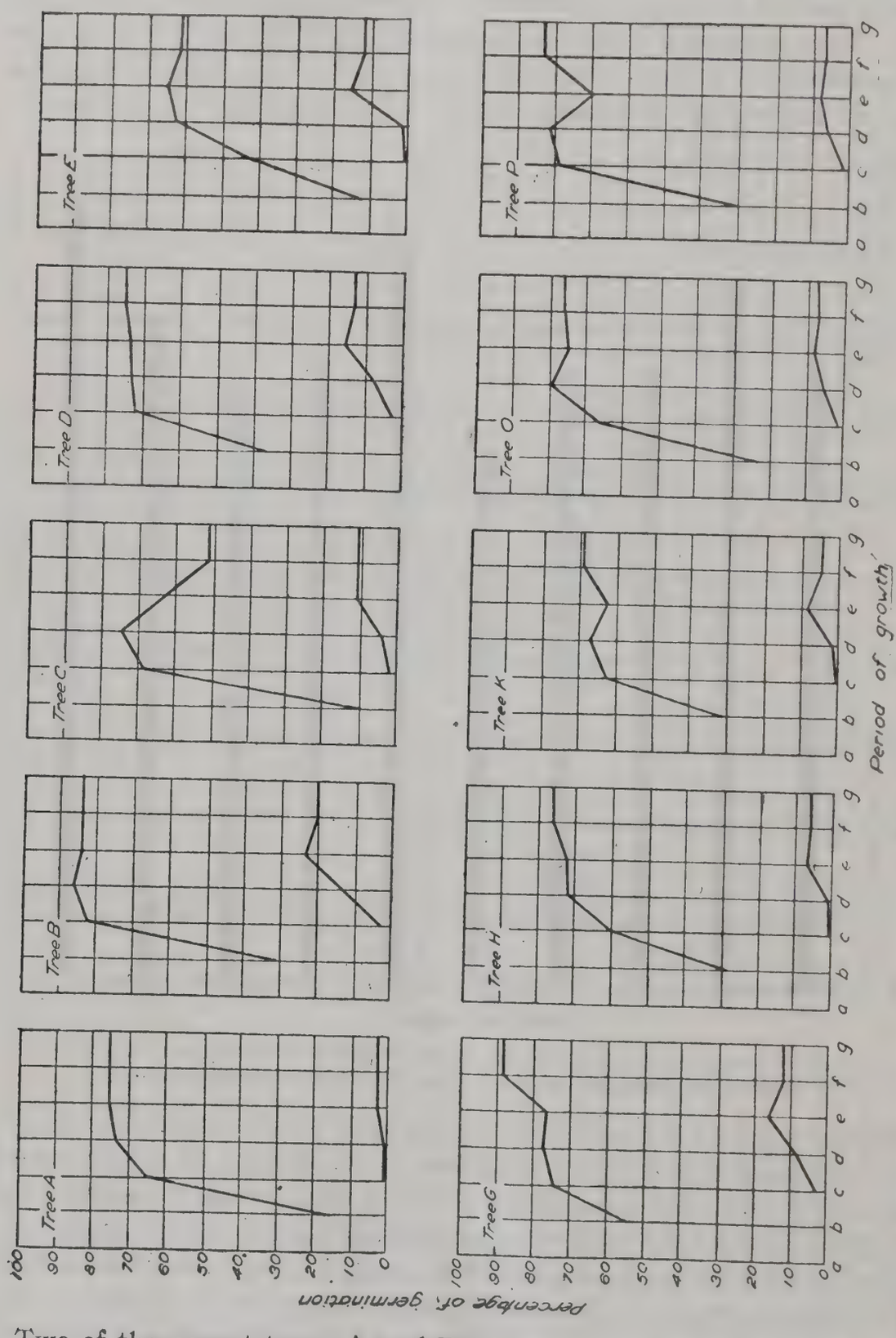


FIG. 5.—Graphs showing rates and percentage of germination of pulp and unpulped camphor seed from 10 parent trees. For explanation, see text.

Two of the parent trees, A and D, show no apparent falling off in the curve of germination for pulped seed; and parent trees A and C show likewise no apparent falling off for unpulped seed. Careful observation

has shown that the burning off of the young seedlings occurred to a greater or less degree throughout all the late winter and early spring and that the burning off was by no means uniform in all the experimental rows. It appears, therefore, that the curves given in figure 5 are not the true curves of germination, but rather the curves of count. However, the true curve of germination follows closely the curve of count with some striking exceptions. As the season advanced a large number of seeds germinated within a short period of time, and as the heat of the sun became more intense a large number of seedlings were burned off. In the case of eight of the trees the burning off of the seedlings between counts reached a point where it included practically all the younger seedlings just pushing up through the ground as well as some of the more tender seedlings of the count previously recorded. Consequently a drop occurred in the curve, which shows as an apparent decrease in the percentage of germination. This explanation is further supported by the fact that somewhat later in the spring, during more favorable weather conditions, germination of more of the seed took place, as shown in Table IV, which caused a rise in the curve of count, especially noticeable in the curves for trees G, K, and P (fig. 5).

In the case of parent tree A, no apparent falling off in the germination of camphor seed is recorded in either the pulped or unpulped seeds; in tree C no apparent falling off is recorded for the unpulped seed; and in tree D no apparent falling off is recorded for the pulped seed. In the case of these trees, A, C, and D, the burning off of the young seedlings which occurred between observations never reached a stage where the total number of seedlings burned off was large enough to cause a decrease to show in the count. For this reason the curve of count for trees A, C, and D, as indicated in figure 5, probably closely coincides with the true curve of germination for the seed from these trees.

CAMPHOR SEEDLINGS BROUGHT TO TRANSPLANTING SIZE

Of more economic importance than the number of seed that germinate is the number of seedling camphor trees which can be brought to transplanting size. Out of 4,800 seeds planted as they came from the tree only 508 seedlings reached a sufficient size for transplanting, whereas from 4,675 seed planted after pulping 3,499 such seedlings were secured. The increase therefore in the percentage of seedlings of transplanting size from the pulped seed over those from the unpulped seed amounted to approximately 600 per cent.

The loss of seedlings due to the burning off by the hot sun is relatively large and has a marked influence on the percentage of seedlings secured. In this experiment 14.5 per cent of the total number of seedlings obtained from the unpulped seed and 5.5 per cent of the seedlings from the pulped seed were burned off. However, the total loss of seedlings from pulped

seed is much less than from unpulped seed, and this lower percentage is due to the advanced growth obtained by the seedlings before the extreme hot weather commenced.

In Plate 20 is shown a nursery bed of camphor seedlings just previous to transplanting on December 1, 1918. In this bed the pulped and the unpulped seed were planted in alternate rows. The small seedlings from the unpulped seed are almost obscured by the alternate rows of large seedlings which were obtained from the pulped seed.

At the time of transplanting, the seedlings had reached the comparative size shown in Plate 21 A. In all instances the pulped seed had produced hardier and more vigorous seedlings, which, when trimmed and cut back as shown in Plate 21 B, were in a better condition to withstand the shock of transplanting. The superior growth of the seedlings from pulped seed was so marked that measurements were taken of 258 seedlings of this lot and compared with measurements taken of the same number of seedlings that came from unpulped seed. The results are given in Table V.

TABLE V.—*Average growth attained by camphor seedlings during the growing period between germination of the seed and transplanting of the seedlings*

Treatment of seed.	Number of seedlings measured.	Average growth.		
		Length of stem.	Length of taproot.	Diameter of crown.
		Inches.	*Inches.*	*Inches.*
Pulp removed	258	13.0	17.7	0.473
Pulp not removed	258	11.0	15.3	.320

If the growth of seedlings from seed on which the pulp remained is considered as 100 per cent, then the increased growth in the seedlings from the pulped seed is: For length of stem, 18.1 per cent; for length of taproot, 15.6 per cent; and for diameter of crown, 47.8 per cent. The latter vigorous growth is of special interest from the commercial point of view, since the loss from transplanting is much less with roots of large diameter than it is with small roots, which are more easily dried out during the period that elapses between the removal of the seedlings from the seed bed and the date of the beginning of growth the following growing season. The increased growth of the seedlings is brought about directly by pulping the seed, since it insures a more rapid germination and gives the plant an early start in the spring and consequently a much longer growing season. The increased growth and vigor reduce to a very low figure the loss through transplanting.

SUMMARY AND CONCLUSIONS

In the season of 1916–17 camphor seeds were planted under various conditions. The experiment was repeated in the season of 1917–18, and commercial conditions were closely approximated.

Removing the pulp from the seed was found to hasten germination by an average of two weeks; it also gave an increase in germination of approximately 525 per cent over that of unpulped seed.

Drying the seed with artificial heat at 55° C. destroyed all vitality.

Soaking the seed in water apparently did not hasten germination; neither did it increase the percentage of seed that germinated.

Soaking the seed in sulphuric acid of 5 per cent by weight destroyed all vitality.

Allowing the seed to ferment and the pulp to decompose in a closed vessel destroyed all vitality.

Seed picked up from the ground showed less vitality than those picked from the tree, but removal of the pulp increased and hastened germination.

A freeze on three successive nights, during which the temperature fell to 26° F., did not destroy the vitality of all the seed; it did, however, impair the vitality and reduce the number of seeds that germinated by approximately 50 per cent.

Seeds planted early in the winter required a longer time to germinate than those planted in midwinter. The former averaged more sturdy trees.

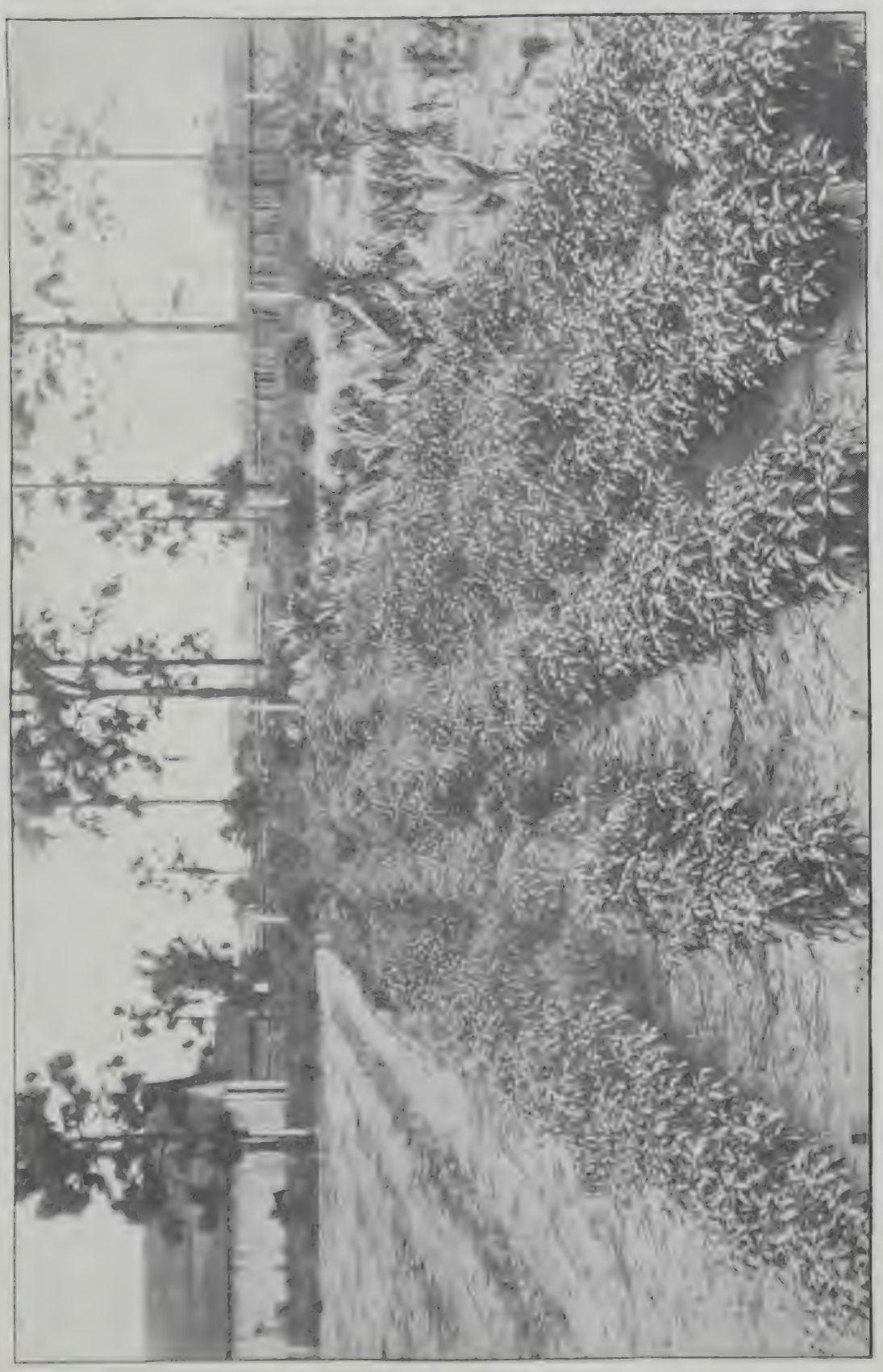
When the pulp was removed and the germination of the seed thus hastened, a larger and more sturdy seedling tree was obtained for transplanting than when the pulp was not removed. The increase in the number of seedlings of transplanting size secured by pulping the seed amounted approximately to 600 per cent.

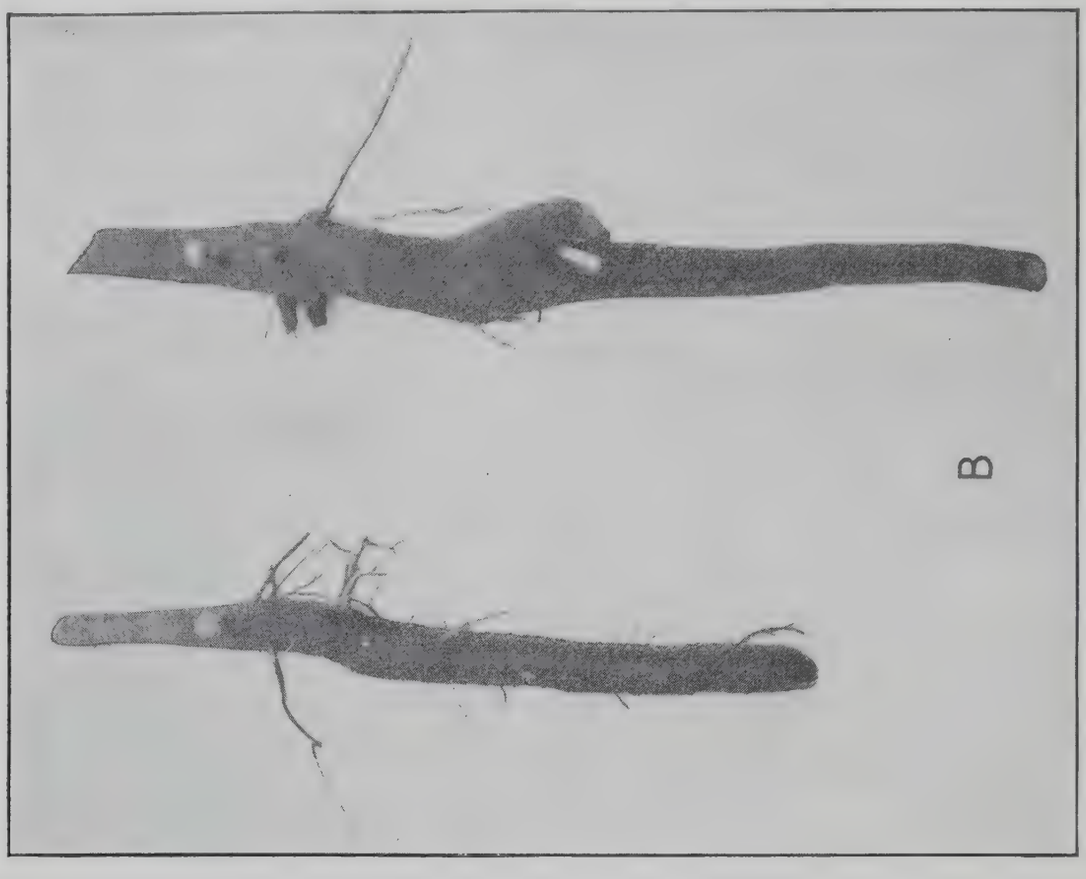
From a commercial point of view, removal of the pulp is desirable even though the labor must all be done by hand. The increased germination and the well-developed trees that result will repay many times the cost of the labor involved.

It is believed that in commercial plantings the removal of the pulp from the seeds will increase the percentage of germination by at least 200 per cent, thus producing 40,000 more seedling trees to each acre of seed bed. This estimate is believed to be very conservative, and even a much greater increase may be expected.

PLATE 20

A camphor seed bed, showing the growth of seedlings from pulped and unpulped camphor seed planted in alternate rows. The seedlings growing from pulped seed have been cut away on one side in order to expose the seedlings growing from unpulped seed, which are otherwise almost completely covered by the luxuriant growth of the former.




B

A

PLATE 21

A.—Camphor seedlings at the time of transplanting. The tree on the left is a representative produced from pulped camphor seed; the one on the right is a representative produced from unpulped camphor seed. Both seedlings are from seed of the same parent tree and both are of the same age from planting of the seed.

B.—Camphor seedlings cut back and trimmed ready for transplanting. These seedlings are the same as those shown in A. The one on the right is from pulped seed.

BACTERIUM ABORTUS INFECTION OF BULLS

[PRELIMINARY REPORT]

By J. M. Buck, G. T. Creech, and H. H. Ladson, *Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

Numerous investigators have called attention to the fact that *Bacterium abortus* agglutinins and complement-fixing bodies can frequently be demonstrated in the blood serum of bulls from abortion-infected herds. Such animals in consequence have frequently been referred to as being systemically infected. While the presence of these bodies constitutes strong evidence that abortion infection exists, or has been present, success has been reported in associating positive reactions with the causative infection in so few instances as to have resulted in a certain amount of speculation regarding the significance of these reactions in male animals.

Literature, it is true, records no great amount of investigative work in connection with bulls suspected of being infected with abortion disease where the object has been the isolation of the causative microorganism from the organs or tissues of the animals or the demonstration of lesions associated therewith.

Schroeder and Cotton [1] in investigating this problem describe two cases that came under their observation. They state that one of the bulls at the time of autopsy showed the presence of an abscess involving the epididymis of one testicle from which *Bact. abortus* was isolated. The other animal was permitted to serve a cow that was considered to be free from abortion disease. Seminal fluid which was recovered from the vagina immediately following the service and injected into numerous guinea pigs produced *Bact. abortus* lesions in one of the experimental animals.

Rettger and White [2] describe endeavors to associate the presence of the infection with positive serum reactions in three cases which they studied. In two of the animals neither abortus infection nor pathological changes could be demonstrated. In the third they call attention to the finding of two abscesses or cysts in the region of the groin, near the point of attachment of the scrotum; but from these abscesses they were unable to isolate the abortion organism, thus failing to obtain bacteriological evidence of the infection.

In view of the positive bacteriological findings of Schroeder and Cotton the present writers were prompted to undertake further investigations,

[1] Schroeder, E. C., and Cotton, W. E. some facts about abortion disease. *In* Jour. Agr. Research, v. 9, no. 1, p. 9–16. 1917.

[2] Rettger, L. F., and White, G. C. infectious abortion in cattle. Conn. Storrs Agr. Exp. Sta. Bul. 93, p. 199–249. 1918. References, p. 246.

involving a considerable number of animals, in an endeavor to ascertain with what frequency abortus infection could be demonstrated in the generative organs of bulls giving positive or suspicious reactions to the agglutination test for this disease, and to determine whether or not pathological changes are commonly associated with such infection.

EXPERIMENTAL PROCEDURE

The procedure employed by the writers consisted in securing blood samples from the animals as they arrived at one of the abattoirs in close proximity to Washington, D. C., for slaughter. No information was available regarding the original source of the bulls or the exposure sustained. Each sample was given a number corresponding to the serial number of a tag that was attached to the animal's ear at the time of bleeding. The blood samples were then taken to the laboratory for the application of the agglutination test. At the time of slaughter, which was usually the following day, those animals giving positive or suspicious reactions were autopsied as carefully as abattoir conditions permitted and the organs of the genital system were secured for further study. Cultural work was depended upon as a means of detecting infection, the medium employed consisting of 3 per cent glycerin infusion agar to which approximately 5 per cent sterile blood serum was added. To reduce the oxygen tension the inoculated tubes were subjected to incubator temperature in closed jars in the presence of fresh cultures of *Bacillus subtilis*.

During the period from December 9, 1916, to July 7, 1918, the agglutination test for abortion disease was applied to 325 mature bulls. Of this number 288 gave negative results to the test. The manner in which the remaining 37 reacted is of considerable interest, inasmuch as the intensity of the reactions appeared to bear some relation to the cultural results.

The manner of applying the test consisted in the making of a 1 to 10 basic dilution of the blood serum. To the four tubes utilized for each case were added 0.4, 0.2, 0.1 and 0.05 cc. of this basic dilution. The amount of test fluid added to each tube was 1 cc.

The vesiculae seminales, vasa deferentia, testes, and epididymides were secured from the 37 bulls whose blood serum showed the presence of *Bact. abortus* agglutinins. From 15 to 20 tubes of medium were utilized for culturing the various organs from each bull. These investigations resulted in the demonstration of the presence of *Bact. abortus* infection in four animals—No. 88, 98, 136, and 409—and in the detection of marked lesions in bulls 98 and 409.

A brief description of the work performed and the findings in these cases follow.

The agglutination reactions of the animals appear in the following table.

TABLE I.—*Results of agglutination tests*

Animal No.	Suspected serum.				Animal No.	Suspected serum.			
	0.04 cc.	0.02 cc.	0.01 cc.	0.005 cc.		0.04 cc.	0.02 cc.	0.01 cc.	0.005 cc.
21	Sl	Sl	−	−	168	+	Sl	Sl	−
84	+	Sl	−	−	177	Sl	−	−	−
86	Sl	Sl	−	−	178	+	Sl	−	−
88	+	+	+	Sl	179	Sl	Sl	−	−
89	Sl	Sl	−	−	189	+	Sl	−	−
98	+	+	+	Sl	198	+	+	Sl	−
103	+	+	+	+	265	+	Sl	−	−
105	+	Sl	−	−	271	+	+	Sl	−
109	+	Sl	−	−	280	+	Sl	−	−
133	+	+	+	+	301	Sl	Sl	−	−
134	Sl	Sl	Sl	−	319	+	Sl	−	−
136	+	+	+	Sl	326	+	+	Sl	−
137	+	+	Sl	Sl	338	Sl	Sl	−	−
143	Sl	−	−	−	348	Sl	−	−	−
146	+	Sl	−	−	409	+	+	+	+
147	Sl	−	−	−	451	+	+	+	−
150	+	+	Sl	−	453	+	Sl	Sl	−
154	Sl	Sl	−	−	454	+	+	Sl	−
165	Sl	Sl	−	−					

+=Complete agglutination.
−=No agglutination.
Sl=Partial clumping of bacteria.

EXAMINATION AND FINDINGS OF BULL 88

February 12, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc. Sl.

February 14, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Fluid of left seminal vesicle turbid in appearance and slightly more excessive in amount than that contained by other organ. No indication of abnormal conditions noted elsewhere.

BACTERIOLOGICAL FINDINGS.—Of the 16 tubes of medium inoculated from the various organs enumerated, after four days' incubation three tubes developed from 40 to 60 colonies of an organism suggestive of *Bact. abortus* and subsequently identified as such. These inoculations were from the left seminal vesicle.

EXAMINATION AND FINDINGS OF BULL 98

March 8, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc. Sl.

March 9, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Marked pathological changes involved the left seminal vesicle. The organ was increased from 8 to 10 times

its normal size. On cross section of the vesicle numerous hemorrhagic areas were observed, as well as a number of necrotic centers, the latter being confined chiefly to the more central portions of the organ. So softened were some of these foci that the necrotic material assumed a semifluid character. The capsule of the organ showed considerable thickening. (See Pl. 22.)

HISTOLOGICAL EXAMINATION.—Sections from the left seminal vesicle showed varying stages of the diseased process, ranging from exfoliation of the epithelial lining of a few of the acini to complete obliteration of the normal glandular structure. There was marked proliferation of the interstitial tissue with round cell infiltration, which was more pronounced immediately surrounding the acini and just beneath the epithelial lining of the acini. In those areas exhibiting the more pronounced pathological changes many of the acini were filled with detached epithelial cells and cell débris. In other areas where the mere outline of the acini could be traced, a homogeneous substance was present, together with more or less granular detritus. There were hemorrhages into and between the acini. Occasionally large areas of degeneration and necrosis were observed. As a result of the inflammatory changes little normal glandular structure was recognized in many of the sections examined (Pl. 24, A and B). Plate 23 A, representing a normal seminal vesicle, is inserted for comparison with Plate 23 B, and Plate 24, A and B.

BACTERIOLOGICAL FINDINGS.—Eighteen tubes of medium were utilized for the culturing of the different organs. The six tubes from the left seminal vesicle after three days' incubation developed from 75 to 150 colonies of an organism that appeared typical of *Bact. abortus*. All tubes inoculated from other sources remained sterile, although incubated for several additional days. Subsequent work with the organism isolated established its identity as *Bact. abortus* and indicated that pure cultures of the organism were isolated in all instances.

EXAMINATION AND FINDINGS OF BULL 136

May 15, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc. Sl.

May 16, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—The right seminal vesicle showed slight enlargement. The fluid contained by this organ presented a turbid appearance. No lesions were elsewhere detected.

BACTERIOLOGICAL FINDINGS.—Five of the 18 tubes of medium inoculated from the different organs developed from 40 to 60 colonies of an organism that was subsequently identified as *Bact. abortus*. These inoculations were from the right seminal vesicle.

EXAMINATION AND FINDINGS OF BULL 409

August 15, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc.+.

August 16, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Left seminal vesicle showed evidence of disease. This organ was approximately twice the size of the right and was incised with considerable difficulty on account of fibrous tissue proliferation. The fluid contained was decidedly turbid. Other organs presented a normal appearance.

MICROSCOPIC EXAMINATION.—Sections from the left seminal vesicle showed marked proliferation of the interstitial tissue with areas of round-cell infiltration. Degeneration and exfoliation of the epithelial cells lining the acini were observed. A few of the acini contained cells and cell detritus; others had been completely obliterated as a result of the inflammatory process. (See Pl. 23 B.)

BACTERIOLOGICAL FINDINGS.—Two of the 20 tubes of medium that were inoculated from the various organs developed colonies typical of *Bact. abortus*. The colonies were few in number and appeared on but 2 of 5 tubes that were sown with material from near the same point. These tubes were from the left seminal vesicle. All tubes inoculated from other sources remained sterile. The infection was subsequently established as *Bact. abortus*.

It has been previously suggested that the intensity of the serum reactions appeared to bear some relation to the cultural results. Of the 37 bulls exhibiting agglutinating properties for a *Bact. abortus* suspension, the blood serum of but 7 caused perfect agglutination of a suspension with 0.01 cc. of the serum. It may be observed that 4 of these 7 animals yielded positive cultural results and that in no instance was the presence of the infection demonstrated in animals when their blood serum failed to cause perfect agglutination with such an amount of serum.

EXAMINATION AND FINDINGS OF GUERNSEY BULL

Since the isolation of *Bact. abortus* infection from the cases previously described, the writers have had an opportunity to demonstrate the presence of the infection and observe lesions in a fifth bull where the isolation of abortion bacteria was carried out under different conditions and where it was possible to obtain a somewhat more complete history in regard to the development of the pathological changes that were associated with the infection.

This pure-bred Guernsey, 8 years of age, was acquired by the present owner in June, 1918, and appeared at the time to be in perfect physical condition.

The writers were informed that during the following January an asymmetrical enlargement of the scrotum was noted. Mechanical injury was

suspected which had prompted the application of fomentations and counterirritants. When the condition failed to respond to this treatment and an area of softening that appeared to involve the left testicle was later detected, a canula had been introduced through which had been evacuated a considerable quantity of a semifluid material. It was furthermore stated that the animal had at times discharged through the urethra a substance bearing some resemblance to that removed by the surgical procedure.

On April 22, 1919, about three months after the swelling was first observed, a sample of blood was secured for the application of the agglutination test for abortion disease. The specimen caused clumping of a *Bact. abortus* suspension with 0.0001 cc. of the serum.

When the animal was examined on the following day with the object of obtaining material for bacteriological work, the enlargement involving the left testicle was found to be four or five times the size of the normal organ. When a needle was passed into its lateral wall, little resistance was encountered after the instrument had been inserted for about 1½ inches. Through the needle were aspirated from 400 to 500 cc. of a gray-colored substance of the consistence of heavy cream. At the same time from 20 to 30 cc. of a turbid fluid were obtained from the urethral opening. This material was secured by exerting pressure on the urethra and by massaging the seminal vesicles through the walls of the rectum. During this procedure it was detected that the seminal vesicles differed markedly in size, enlargement of the right organ being pronounced.

CULTURAL RESULTS.—Eight tubes of serum agar were inoculated with the semifluid substance aspirated from the interior of the enlargement involving the diseased testicle. Numerous dilutions were made of the fluid recovered from the urethra with physiological salt solution, and serum-agar tubes were sown with these dilutions. When the tubes were examined after six days' incubation one colony of abortuslike appearance was observed on one of the tubes from the substance obtained by aspiration. The infection was later established as *Bact. abortus*. Further inoculations of medium with like material resulted in the isolation of additional abortus colonies, although fewer in number than were anticipated from the extent of the lesions. No *Bact. abortus* was isolated by cultural methods from the material secured from the urethra, but excessive contamination made these results inconclusive.

On May 9, or about two weeks after the condition was diagnosed as abortus infection, an opportunity was afforded for the making of a more thorough examination of the diseased process involving the external genitals and for further bacteriological work, for the affected testicle with its coverings were at this time removed and forwarded to the Pathological Division.

The weight of the mass of tissue was 5½ pounds. On section it was found to consist of an outer wall or capsule of from 1½ to 2 inches in thickness. This abnormal structure had evidently resulted from proliferative changes involving mainly the connective tissue coverings of the testicle. Firmly embedded in this external layer could be distinguished areas of tissue that upon microscopic examination were identified as epididymis that had undergone severe inflammatory changes. The cavity formed by this dense fibrous wall contained a considerable quantity of a grayish-colored, semifluid material identical with the substance previously obtained by aspiration. Floating free in the cavity was also a mass of tissue that was recognized as the remains of the testicle, it having the same general form although somewhat reduced in size. Blood vessels no longer communicated with the organ, and the serous membranes which normally envelop it had seemingly been entirely obliterated. The close resemblance existing between the semifluid substance and softened portions of the testicle strongly indicated that the organ was undergoing liquefaction necrosis.

MICROSCOPIC EXAMINATION.—The thick wall surrounding the testicle consisted largely of dense fibrous tissue with a certain amount of round-cell infiltration. Different portions of the epididymis which were embedded in this mass showed extensive interstitial proliferation, which had resulted in a wide separation of the tubules. Chronic inflammatory changes were noted in sections from the testicle proper. Many tubules were surrounded by zones of round-cell infiltration. There was exfoliation and more or less disintegration of the epithelium lining the tubules, causing the latter to be largely occupied by cell débris. Advanced degenerative changes, verging on necrosis, were observed in all the sections examined, the peripheral portion of the organ exhibiting little more than a mere outline of the testicle structure.

BACTERIOLOGICAL FINDINGS.—Tubes of serum agar that were inoculated with the exudate at this time developed numerous colonies of an organism that was identified as *Bact. abortus*.

It has been suggested by writers on abortion disease that *Bact. abortus* infection when acquired by bulls remains active for a comparatively brief period, the resistance offered being sufficient for its destruction. The encountering of a considerable number of animals giving slight agglutination reactions and the isolation of abortus infection from only a small percentage of the bulls cultured would tend to strengthen the theory that the infection may commonly terminate in this manner. On the other hand the extensive pathological changes and the chronic character of the lesions exhibited by three of the five bulls where abortus infection was demonstrated suggest that it may be unwise to assume that long-standing cases of infection never exist.

CONCLUSIONS

Bact. abortus infection may involve organs of the generative apparatus of bulls, producing chronic inflammatory changes.

Of the generative organs, the seminal vesicles appear to furnish the most favorable site for the lodgment and propagation of abortion infection.

The presence of *Bact. abortus* infection in bulls appears to be more strongly indicated by relatively marked than by slight reactions to the agglutination test for this disease.

PLATE 22

Photograph of normal and diseased seminal vesicles of bull 98, showing the marked increase in size and the gross pathological changes of one of the organs.

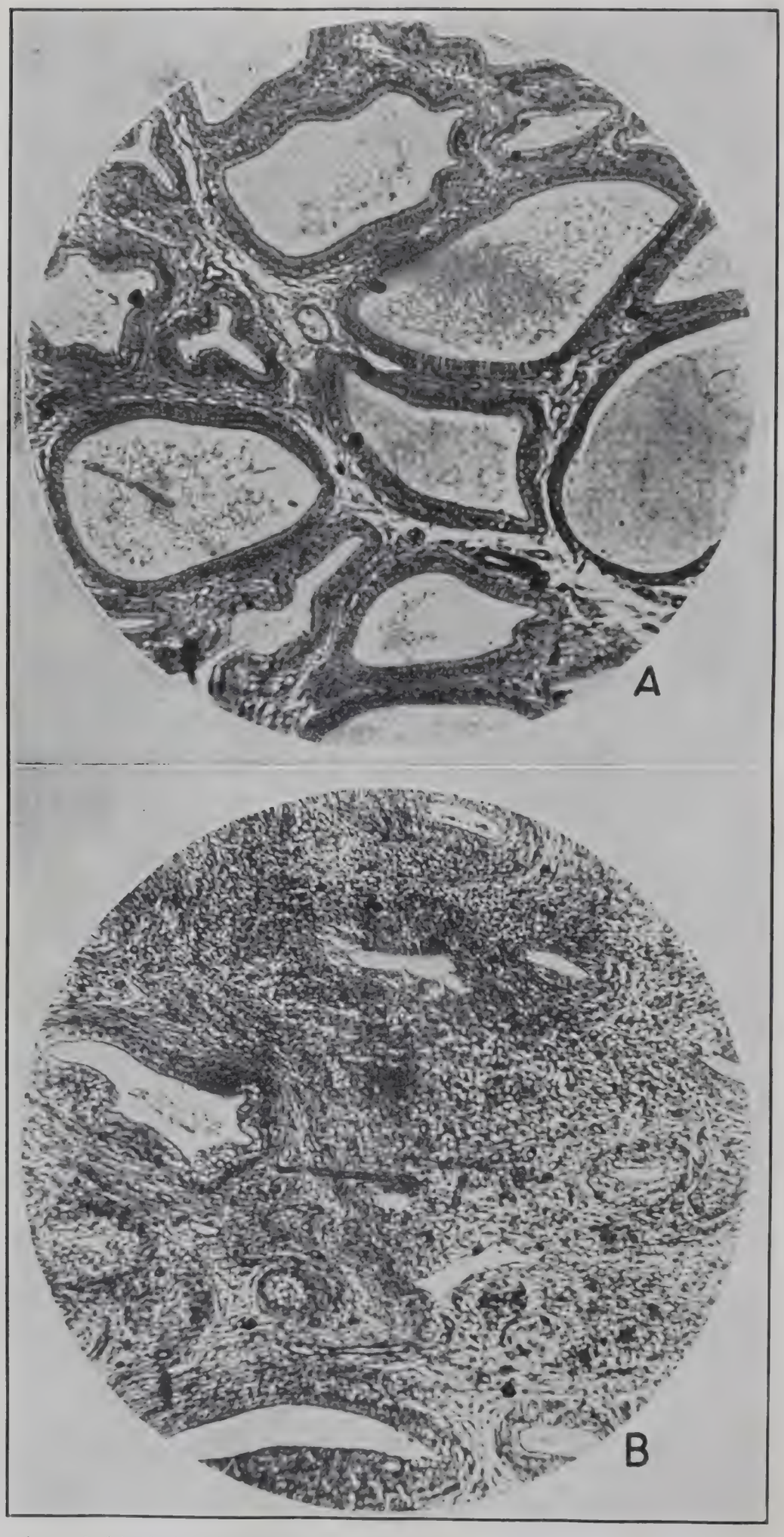

A
B

PLATE 23

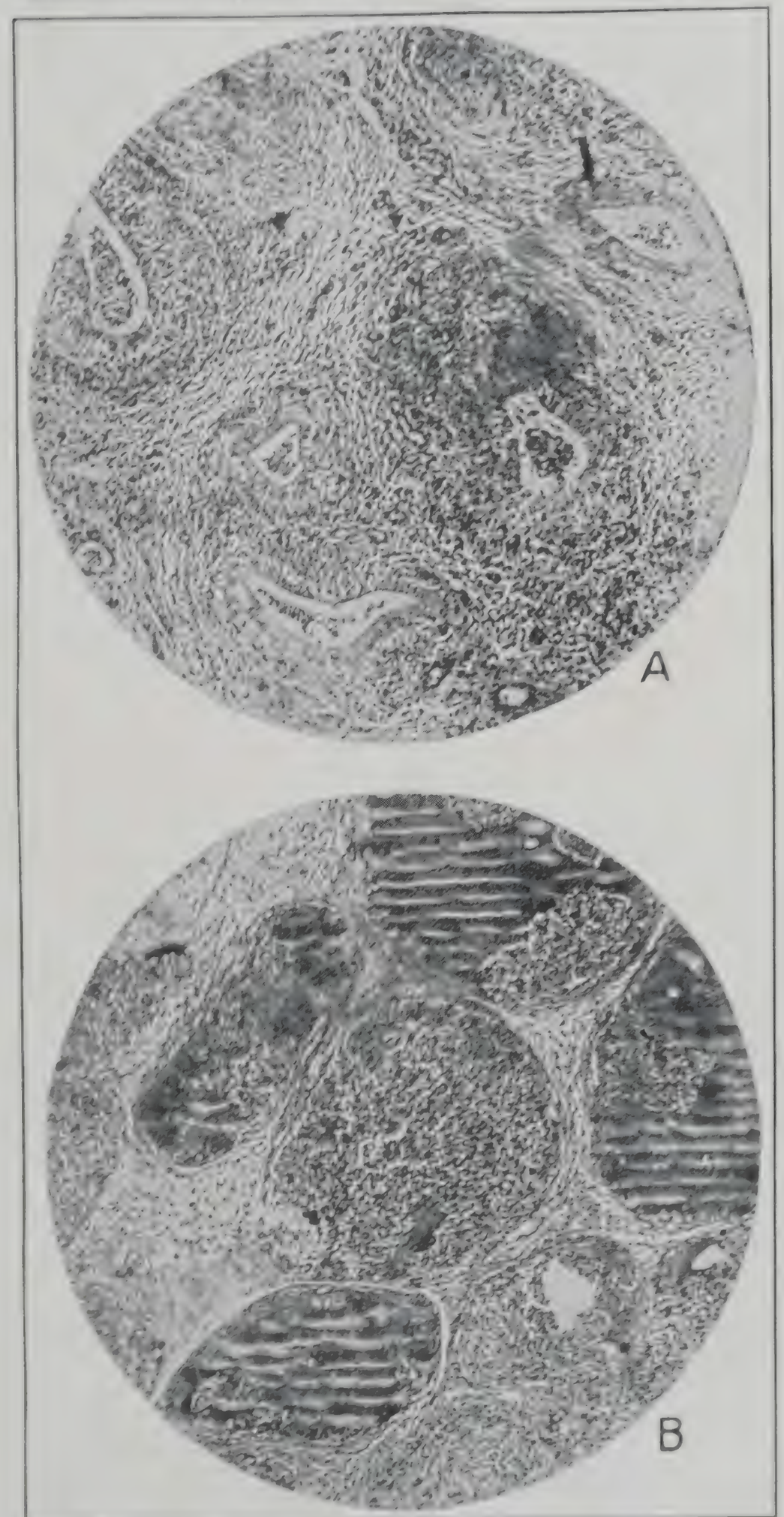
A.—Photomicrograph of a section from a normal seminal vesicle of bull. × 92.

B.—Photomicrograph of section from seminal vesicle of bull 409, showing inflammatory changes. × 92.

PLATE 24

A.—Photomicrograph of section from seminal vesicle of bull 98, showing tissue proliferation and exfoliation of epithelium lining acini. × 92.

B.—Photomicrograph of section from seminal vesicle of bull 98, showing advanced pathological changes with cell degeneration and necrosis. × 92.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INVESTIGATIONS ON THE MOSAIC DISEASE OF THE IRISH POTATO[1]

[PRELIMINARY PAPER]

By E. S. SCHULTZ,[2] *Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture,* DONALD FOLSOM, *Assistant Plant Pathologist, Maine Agricultural Experiment Station,* F. MERRILL HILDEBRANDT, *Junior Chemist, and* LON A. HAWKINS, *Plant Physiologist, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The economic importance and wide distribution of the mosaic or "calico" disease of tobacco (*Nicotiana tabacum* L.), as well as its distinguishing characteristics, have been a matter of common knowledge among pathologists and practical growers for many years. The fact that mosaic occurs also on certain others of the Solanaceae is well recognized, but it has been known for only a comparatively short time that the Irish potato (*Solanum tuberosum* L.) is subject to a similar malady.

As will be shown, potato mosaic, although more common and apparently more destructive in certain sections of the United States than in others, is widely distributed in North America. While the data regarding it which have so far accumulated are necessarily limited, there is a tendency among those pathologists who have given the subject special study to regard it as a disease of great economic importance. The results of the studies described in this paper, chiefly those which throw light on the means of transmission of the disease, are made more significant by the fact that they were obtained in four different laboratories, partly through collaboration and partly as the result of independent work.

[1] This paper was read at the conference of potato pathologists on Long Island, June 26, 1919. An abstract was published in Phytopathology.

The investigations were conducted as a cooperative project between the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry and the Department of Plant Pathology of the Maine Agricultural Experiment Station.

[2] The authors wish to acknowledge their indebtedness to Dr. H. A. Edson and Dr. W. J. Morse for helpful suggestions and criticism of the manuscript and to Dr. Joseph Rosenbaum, Mr. M. Shapovalov, and Mr. G. B. Ramsey for assistance in furnishing material and collecting data.

GEOGRAPHICAL DISTRIBUTION OF POTATO MOSAIC

Orton (*9, p. 42*)[1] in 1911 first observed potato mosaic in a field at Giessen, Germany, where it was very common on some varieties. The following year it was found to be prevalent in the potato fields in northern Maine but was not found in Wisconsin, Minnesota, Colorado, and other western states during an extended survey made in 1912 and 1913. In 1913 Melchers (*6, p. 153*) observed symptoms of this disease in the greenhouse on potato plants from tubers from New York. More recently Wortley (*12*) reported it as very prevalent on the Bliss Triumph variety in Bermuda and on Long Island, and Murphy (*8*) said that the disease occurred to a considerable extent in New Brunswick and to a less extent in western Canada. In 1917 and 1918, collaborators for the Plant Disease Survey reported it from the following states: Alabama, Arkansas, Connecticut, Delaware, Florida, Georgia, Kentucky, Louisiana, Maine, Massachusetts, Michigan, Minnesota, New Hampshire, New York, North Dakota, Ohio, Oregon, Texas, Vermont, Virginia, and Wisconsin. From these reports it is apparent that potato mosaic occurs rather generally throughout the United States.

Although potato mosaic, named as such, has been reported for the first time within the last decade, the following statement made by Johnson (*4*) before the middle of the nineteenth century is of interest. In a description of a potato disease which seems to have somewhat resembled mosaic he says:

> The stem is unbranched, brownish green or mottled, and here and there sprinkled with rusty spots, which penetrate to the pith, so that it is not white but rust colored or sometimes black. The upper surface of the leaves is not as smooth as is usual in the case with potato leaves but *rough, wrinkled, or curled. The leaves are far more sessile than usual, and are not of a uniform brownish or dark green color, but spotted.*[2]

Johnson further says that this trouble can be produced by repeated removal of the sprouts before planting.

EFFECTS UPON YIELD

The yield from affected plants is less than that from healthy vines of the same variety. Orton (*9, p. 42*) as the result of an experiment with Green Mountain potatoes in northern Maine reports a difference in yield of 22 per cent between 80 healthy and 80 diseased hills. Wortley (*12*) states that 200 healthy Bliss Triumph hills yielded more than twice as much as 200 diseased hills of the same variety and that mosaic of potatoes in Bermuda frequently causes a reduction in yield of from 10 to 75 per cent. Murphy (*8*) compared 682 diseased Green Mountain hills, scattered over 11 plots, with the same number of healthy hills growing adjacent to the diseased hills. He found the yield of the former to be

[1] Reference is made by number (italic) to "Literature cited," p. 272–273.

[2] Italics in this quotation are supplied by the writers.

but 58 per cent of the latter and concluded that in New Brunswick, Canada, the yield is reduced about $1\frac{1}{5}$ bushels for every 1 per cent of mosaic present. Reduction in yield reported by collaborators of the Plant Disease Survey (*11*) ranges from 5 to 30 per cent. Comparative results secured by the writers in northern Maine will be discussed later in connection with the questions of hill selection and roguing.

The preceding statements refer to the comparative yields of healthy and entirely diseased lots and so may seem to be somewhat inapplicable to conditions where a large number of the plants are not diseased and where these may possibly be able to make up for the deficiency of affected plants by making better growth at their expense. However, the writers have found that often, in the absence of any control measures, a healthy lot of a susceptible variety will show symptoms of the disease in some hills the next year after being grown near to diseased stock and will thereafter from year to year have a larger percentage of hills affected.

APPEARANCE OF THE DISEASED PLANTS

Some of the symptoms will be described here, although the subject has already received considerable attention (*6*, *7*, *8*, *10*). On Green Mountain or Bliss Triumph potatoes, the leaves of affected plants are characterized by mottling (Pl. A; B; 25), which is produced by the presence of light green areas on the foliage. These areas may occur on any part of the leaf; they may include or adjoin sections of the larger veins or not come in contact with them. The light green patches vary greatly in shape, being punctate, elongate, circular, angular, and irregular. Considerable variation in the degree of paleness may be seen even in the same small discolored patch, from a barely discernible fading of the green to an almost pure yellow. The abnormal spots differ in distinctness of outline, usually in proportion to the degree of discoloration. Their dimensions seldom exceed a few millimeters. Their frequency varies, usually becoming greater as the disease progresses and thus giving to the general appearance of the leaves a much lighter color than that of healthy foliage. In the more severely affected plants the foliage may become spotted with brown flecks of dead tissue. Furthermore, in the more advanced stages the foliage presents a characteristic crinkled or corrugated appearance. In these stages the diseased plants are frequently dwarfed because the stems, the leaf petioles, and leaf blades are considerably shortened or reduced in size.

The symptoms as described above are not so marked in certain other varieties—for example, in Blue Victor, Early Rose, Irish Cobbler, Pearl, White Bliss, Carmen, Early Dix, Netted Gem, Peach Blow, Portuguese Purple, and Spaulding Rose. In the first five named, decided rugosity is a characteristic of the disease.

So far no symptoms have been discovered by which mosaic can be recognized in the dormant tubers; nor has any effect upon the percentage of germination or the time of blossoming been observed, although premature death may occur.

The presence of mottling on the leaves is apparently modified by climatic conditions. It was found by planting a part of the same affected stock and strain in northern Maine and Colorado, that, whereas distinct mottling occurred in northern Maine, none whatever could be detected on the stock in Colorado during the same season. Similar tests were made at Washington, D. C., and in northern Maine; and although some mottling was noted at Washington there were a number of doubtful cases, while the same stock in northern Maine showed very distinct mottling. Melhus (7) found that progeny of plants which were mottled in northern Maine did not show such symptoms in Iowa but showed symptoms of "curly dwarf."

For three successive seasons a number of lots of mosaic and healthy seed potatoes have been divided and planted at the two experimental farms of the Maine Agricultural Experiment Station. One of these farms is located in the northeastern and the other in the southwestern part of the state. Usually the part of a lot grown in southwestern Maine showed considerably less mottling than the part grown in northeastern Maine, while the reverse has never been noted. In two out of the three seasons these differences have been very marked. On the other hand, when the same lots which showed practically entire absence of mosaic mottling in one location—in southwestern Maine—one season were removed to the other and planted the following season, the mottling again appeared in marked degree.

TRANSMISSION STUDIES

TRANSMISSION BY TUBERS

Orton (*9*) cites a preliminary experiment and suggests the probability of tuber transmission. Wortley (*12*) found that all tubers from affected plants produced foliage with mottled leaves. Stewart (*10*) says that mosaic is transmitted through the tubers. As pointed out before, Melhus (7) showed that, under Iowa conditions, plants from diseased tubers might not exhibit the mottling of the leaves but might show a dwarfing and curling of the foliage similar to "curly dwarf." Murphy (*8*) says, "Mosaic is perpetuated by planting the tubers from diseased hills." These conclusions are confirmed by evidence which has been secured by the writers and which will be presented later in connection with the questions of hill selection and roguing.

TRANSMISSION BY GRAFTING

Experiments were carried out in the winter of 1916–17 at Washington, D. C., to see if it were possible to transmit the disease by grafting. In these experiments two methods of grafting were followed, the cleft-graft and the inarch. According to the first method the top of a young, apparently healthy, potato plant was removed, the base sliced down to a thin wedge and grafted in the place of the top of a diseased plant. The scion was held in place by winding with adhesive tape. Of six plants grafted in this way that grew well, all the scions showed evidence of the disease (Pl. 26, A). Four of the plants from which the scions were taken remained apparently healthy. The other two showed evidences of the disease. Grafts were made according to the inarch method by placing a healthy and a diseased plant side by side, slicing away a thin layer of the outer tissue of the stem, bringing the cut surfaces in close contact, and fixing them by wrappings of adhesive tape. After the plants had remained in contact for several days the stem of the healthy plant was cut below the point of attachment and the top of the diseased plant removed. In three grafts made in this way the scion of one became diseased while the parent plant remained healthy. The other two were doubtful. This last-mentioned method of grafting seemed not to be adapted to potato plants because, unless maintained in a very humid atmosphere the scions wilted. However, Güssow (*3*) in 1918 by inarching a mosaic shoot on a healthy one found that no mosaic symptoms formed on the foliage of the sound plant but that tubers from it produced mosaic plants.

The results obtained in these preliminary experiments were corroborated by a number of experiments in the field in 1917, the results of which are shown in Table I. In this series no attempt was made to control aphids, nor were any observations made, after grafting, on the plants which supplied the scions. However, these plants were from 4 to 6 inches high and free from mottling at the time of grafting.

TABLE I.—*Grafts of potato vines, Presque Isle, Me., 1917*

Date.	Variety.	Graft.	Number grafts.	Number mottled.	Number nonmottled.	Number doubtful.	Percentage mottled.
August	Bliss Triumph...	Healthy scion on affected stock.	17	11	2	4	64.71
Do..	Green Mountain ..	.....do.....	17	10	5	2	58.82

During the summer of 1918 grafting experiments were continued in northern Maine. Although more than 100 grafts were made, relatively few of these made sufficient growth, 4 to 12 inches, to show distinct

mottling. In order to study the behavior of the plants from which the scions were taken, these plants as well as the scions and stocks were labeled. Their performance is indicated in Table II under the heading of "Condition of parent vine." The grafts were made when the plants were from 4 to 10 inches in height. In the majority of cases the cleft-graft method was used. After the insertion of the scion the contact between scion and stalk was effected by wrapping tightly with adhesive tape. The performance of these grafts is recorded in Table II.

TABLE II.—*Grafts of potato vines, Presque Isle, Me., 1918*

Date.	Variety.	Graft.	Condition of parent vine.	Number of successful grafts.	Number of grafts mottled.	Number of grafts non-mottled.
July 6 to Aug. 17.	Bliss Triumph....	Healthy scion on diseased stock.	Healthy to end of season	14	14	.....
Do.....	Green Mountain..	.....do.....	...do.....	19	19	.....
Do.....	Bliss Triumph...	Healthy scion on healthy stock.	...do.....	6	.....	6
Do.....	Green Mountain..	.....do.....	...do.....	5	.....	5
Do.....	...do.....	Affected scion on healthy stock.	.....	2	2	.....
Do.....	Bliss Triumph ...	.....do.....	.....	3	3	.....

These results indicate plainly that distinct mottling of the healthy scions grafted upon diseased stocks had developed by the end of four or five weeks, whereas no mottling developed on either the parent plants or the healthy scions grafted upon healthy stocks. (See Pl. 27, A, B.) A few new shoots from stocks supporting affected scions showed mottling, but since only a small number of these grafts were made the results are inconclusive.

In the winter of 1918–19, 61 Green Mountain grafts were made at Orono, Me., by means of the cleft-graft method already described. Of the 50 which survived, 14 consisted of healthy scions on healthy stocks and remained entirely healthy for from 43 to 82 days, 9 making new growth from the stock and 1 from the scion; 15 consisted of healthy scions on mosaic stocks; and 7 of these, or 41 per cent, developed mosaic on the scion in from 21 to 44 days, although the plants from which the scions came remained healthy. In the 7 mosaic scions there was usually a continuation of leaf expansion, and the mosaic symptoms developed in the youngest leaves. The scions which remained healthy usually showed no good growth. Of 21 grafts consisting of a mosaic scion and a healthy stock, the one whose stock produced the most new growth showed

much wrinkling and some mottling on this new growth; 3 other stocks showed wrinkling only; and the rest remained healthy, even in the rather poorly developed new shoots.

TRANSMISSION BY PLANT JUICES

Attempts were made to inoculate tubers of the Green Mountain and Bliss Triumph varieties with juice from diseased plants. In these inoculation experiments the method followed was to divide the potato in half longitudinally, make a cavity in one piece, fill this cavity with the juice from the crushed stems and leaves of the diseased plants, and then plant this treated piece. The other half of the potato was planted in a separate pot as a control. In the first experiment, with four Bliss Triumph and four Green Mountain tubers, all the stalks from four of the inoculated portions of these tubers were typical mosaic plants. One of the control plants, corresponding to one of the inoculated portions which developed mosaic, also showed the disease. These experiments were repeated a number of times with larger numbers of tubers, but only occasionally did the inoculation appear to be successful. In all of the transmission experiments it has been difficult to secure seed-tuber lots which were absolutely free from mosaic contamination, so it was to be expected that occasionally both the inoculated and uninoculated parts of the same tuber would produce diseased plants. On the other hand, the uninoculated controls remained healthy in some experiments where the inoculated seed piece produced a mosaic plant, while the converse did not occur. Hence the evidence secured is presumptive that the disease can be transmitted by inoculating seed tubers with juices of affected plants.

In northern Maine during the season of 1918, 50 hills of apparently healthy potato plants of the Green Mountain variety were treated with the filtered and unfiltered extracts from diseased tubers and leaves. These juices were applied by means of painting upon rubbed, bruised, or slashed leaves, and by hypodermic injection into the petioles. The plants at the time of this treatment, July 9 and 10, were about 12 inches tall and in actively growing condition. Observations on July 20 and on August 17 indicated that no treated plants had developed mottling but appeared like the controls, which were treated with water. In order to note whether this treatment of the foliage had any effect upon the tubers, progeny of these hills was reserved for study in 1919.

On November 23, 1918, in a preliminary experiment in the greenhouse at Washington, D. C., juices extracted from potato vines were transferred to foliage of the Bliss Triumph variety. This operation was performed several times in the course of a month, the first inoculation being made when the plants were 3 to 6 inches high. By December 20, 1918, fully 30 per cent of the inoculated plants showed mottling on the

youngest leaves. It was noted also that this mottling occurred only in connection with two very similar treatments. In view of these suggestive results, a similar experiment with the more promising of the methods employed in November, 1918, was begun February 22, 1919.

In this experiment healthy plants from 17 different tubers of the Green Mountain variety were inoculated according to the methods indicated at the foot of Table III. At the time of planting, each of the 17 tubers was halved lengthwise, so that for each treated plant an untreated control plant of the same tuber was obtained. The halves of each tuber were designated respectively x and y and with the same number. Each half tuber was planted in an 8-inch pot.

At the time of the first inoculation, the height of the plants varied from 2 to 6 inches, and the number of shoots to each half tuber varied from two to seven. As shown in Table III, plants from the tuber halves 472x, 483x, 473y, 484x, 471y, and 485y were treated with juices from healthy plants according to the methods indicated and served as control to the plants treated similarly but with juices from mosaic potato vines. The remaining 11 plants, from as many tuber halves, were treated with juices from mosaic foliage. All juices were taken from vines of the Green Mountain variety.

The performance of the treated and untreated plants is noted in Table III. Number 472y represents the untreated plant and 472x the treated plant developed from the same tuber. At this time observations on foliage of the plants treated according to method 3 with juices from mosaic plants indicated that no mottling had developed. This method failed to produce mottling in the November experiment also. However, with method 5 and with method 7 seven different plants had developed new leaves since March 22; and five of them, or 71 per cent, showed distinct mosaic mottling on the younger leaves formed after the time of the last inoculation on March 22 (Pl. 28, A). The first mottling on any of these plants was noted on March 25. On examination March 25 and April 3, 1919, no mosaic mottling was found on either the old or newly formed leaves in any of the controls, treated or untreated (Pl. 27, A, B). All the plants in this experiment were free from aphids.

TABLE III.—*Foliage inoculation with the mosaic of Irish potato at the greenhouse, Washington, D. C., 1919*

No. of plant.	Method of inoculation.	Condition of foliage at time of first inoculation.	Height in inches at first inoculation.	Number of shoots.	Date of examination.	Condition.	No. of control.	Condition.
472x	Juices from healthy plant. Method 3.[a]	Healthy	3 to 5	3	Apr. 3, 1919	No mottling	472y	No mottling
483x	do	do	2 to 6	4	do	do	483y	Do.
473y	Method 5	do	3	4	do	do	473x	Do.
484x	do	do	3	3	do	do	484y	Do.
471y	Method 7	do	2	2	do	do	471x	Do.
485y	do	do	1 to 2	3	do	do	485x	Do.
475x	Juices from mottled plant. Method 3.	do	3	4	do	do	475y	Do.
492x	do	do	4	4	do	do	492y	Do.
487x	do	do	4 to 6	3	do	do	487y	Do.
489y	Method 5	do	4	4	do	No new leaves since last inoculation	489x	Do.
490x	do	do	5	2	do	Distinct mottling on new leaves of two stalks. Mottling found since last inoculation.	490y	No mottling.
491x	do	do	3 to 5	3	do	Distinct mottling on two shoots found since last inoculation. No young leaves on other shoot and no mottling.	491y	Do.
494x	do	do	2 to 4	7	do	No mottling	494y	No mottling. Only one stalk with new leaves formed since Mar. 15.
488y	Method 7	do	3 to 6	2	do	Distinct mottling on young leaves of both shoots. Mottling noted since Mar. 25.	488x	No mottling.
495y	do	do	3	2	do	Distinct mottling on young leaves of one shoot only. Other shoots no new leaves since Mar. 15.	495x	Do.
498x	do	do	1 to 4	5	do	Distinct mottling on few leaves on one shoot only. Two branches of this stalk with newly formed mottled leaves.	498y	
499x	do	do	2	2	do	No mottling. Young leaves beginning to form	499y	

[a] Method 3: Injection of filtered juices into shoots cut back to within 2 inches from soil. Method 5: Application of unfiltered juices to young leaves and subsequent rubbing and crushing parts of these leaves between fingers. Method 7: Application of leaves to those of plant, both sets of leaves being crushed and rubbed together.

TRANSMISSION BY APHIDS

The fact that plant diseases are frequently carried by insects is well recognized. In this connection the work of Allard (*1, p. 626*) on the mosaic of tobacco is of special interest. This writer showed that the virus of tobacco mosaic is readily carried by the common green peach aphis, or spinach aphis (*Myzus persicae* Sulz.). More recently McClintock and Smith (*5*) have demonstrated that the spinach-blight, which apparently is a virus disease, also is transmitted by plant lice, the pink and green potato aphis (*Macrosiphum solanifolii* Ashmead) and the spinach aphis both acting as carriers. Doolittle's work (*2*) with cucumber mosaic is also worthy of mention in this connection.

FIELD EXPERIMENTS WITH INSECT CAGES

From findings of these writers it has seemed possible that the mosaic of potato might be spread by some insect. To study this question an attempt was made to grow plants in the field under cages that were supposedly insect-proof. These were 22 by 30 by 36 inches, covered with cheesecloth, one side being arranged so that it could be opened—a type that was used also by McClintock and Smith (*5, Pl. 5 and 6*). Potatoes were planted about 14 inches apart, so arranged that one cage covered two hills. During the season of 1917 at Presque Isle, Me., potato plants were grown throughout the season under these cages and observations made from time to time on their condition as regards mosaic. It was found that the percentage of mosaic in the cages was practically the same as that to be found in the same stock planted in the adjoining plots. However, since the disease may be acquired in one season without showing the symptoms until the tubers develop their shoots the following season, it was necessary to continue the comparison through 1918. It was then found that not more than 5 per cent of the tubers from healthy plants caged in 1917 were mosaic, the lowest season-to-season percentage of increase on record for lots grown on the experimental plots.

Tubers from plants grown under the cages in 1917 and not showing the characteristic mottling of the disease during the season were selected for planting in the cages in 1918. On account of the poor quality of the cheesecloth obtainable in the second season the cages were not insect-proof; and within them there were found, at the end of the season, considerable numbers of aphids as well as some insects of other kinds. However, since the dispersal of aphids probably was checked more or less by the cages, tubers were reserved for the 1919 season for comparison with uncaged lots.

GREENHOUSE EXPERIMENTS WITHOUT INSECT CAGES

Since greenhouse conditions are more favorable to the control of aphids, experiments with the pink and green potato aphis were conducted in the greenhouse at Washington, D. C., during the winter of 1917–18. The insects were allowed to develop on stock of the Bliss Triumph variety which during the preceding summer had been rogued in the field in northern Maine—that is, had the plants showing mottling eliminated from the stock. However, as Tables IV and V show, about 22 per cent of the plants developed mottling on January 28, 1918, when they were from 2 to 6 inches tall. From these affected plants the aphids were permitted to disperse to the neighboring, apparently healthy plants; and in addition on March 5 artificial transfers of aphids from diseased to nonmottled plants were made on fully a dozen different plants. By March 19, 1918, it was noted that many of the plants infested with aphids had developed a crinkling and mottling, on the newly formed leaves only, very similar to mosaic mottling (Pl. 29, A). The number of such mottled plants increased so that by April 6, 1918, 50 per cent of the plants showed mottling. On the other hand, only 15 per cent of the remainder of this 1917-grown stock were diseased when grown at Presque Isle in the season of 1918. This 15 per cent, as well as the 22 per cent which first showed mosaic in the greenhouse experiment described above, undoubtedly were progeny of hills that had become diseased in 1917 in spite of the roguing. The increase to 50 per cent seems to be explained best by the dispersal of the aphids from the diseased plants. Moreover, the percentage of plants to which the aphids transmitted the disease in this experiment was really 100, inasmuch as all plants, whether or not eventually becoming mottled in 1917–18, produced progeny which was decidedly mottled in the winter of 1918–19. (See Table IV, "Performance of second generation.") That is, all the tubers which were saved from nonmottled plants, as well as all tubers from the mottled plants, produced mosaic vines in the following winter when planted in the same greenhouse with no aphids present.

TABLE IV.—*Effect of aphids on mosaic of potato at greenhouse, Washington, D. C., 1918–19*

[Bliss Triumph variety]

Pot No.	Condition of plants on[a]—				Performance of second generation, inspected Jan. 4, 1919.
	Jan. 28, 1918.	Mar. 1, 1918.	Mar. 19, 1918.	Apr. 6, 1918.	
101a	H[b]. . . .	H, A. . .	H, A. . .	H, A.	Mm.
101b	H.	H, A. . .	H, A. . .	H, A.	Mm.
102a	H.	H, A. . .	M, A. . .	M, A.	Mm.
102b	H.	H, A. . .	M, A. . .	M, A.	Mm.
103a	M.	M, A. . .	M, A. . .	M, A.	
103b	M.	M, A. . .	M, A. . .	M, A.	
104a	H.	H, A. . .	M, A. . .	M, A.	Mm.
104b	H.	H, A. . .	M, A. . .	M, A.	Mm.
105a	M.	M, A. . .	M, A. . .	M, A.	
105b	M.	M, A. . .	M, A. . .	M, A.	
106a	H.	H, A. . .	H, A. . .	M, A.	Mm.
106b	H.	H, A. . .	H, A. . .	H, A.	Mm.
107a	H.	H, A. . .	H, A. . .	H, A.	Mm.
107b	H.	H, A. . .	H, A. . .	H, A.	Mm.
108a	H.	M, A. . .	M, A. . .	M, A.	
108b	H.	M, A. . .	M, A. . .	M, A.	
109a	M.	M, A . .	M, A. . .	M, A.	
109b	M.	M, A. . .	M, A. . .	M, A.	
110a	H.	H, A. . .	M, A. . .	M, A.	
110b	H.	H, A. . .	M, A. . .	M, A.	
111a	H.	M, A. . .	Dead. .	. . . . .	
111b	H.	H, A. . .	H, A. . .	H, A.	Mm.
112a	H.	H, A. . .	M, A. . .	Dead.	
112b	H.	H, A. . .	M, A. . .	. . . . .	Mm.
113a	M.	M, A. . .	M, A. . .	Dead.	
113b	M.	M, A. . .	M, A. . .	. . . . .	
114a	H.	H, A. . .	H, A. . .	Dead.	
114b	H.	H, A. . .	H, A. . .	. . . . .	
115a	H.	H, A. . .	H, A. . .	H, A.	
115b	H.	H, A. . .	H, A. . .	H, A.	
116a	H.	H, A. . .	M, A. . .	Rapidly maturing.	Mm.
116b	H.	H, A. . .	M, A. . .	. . . . .	Mm.
117a	M.	M, A. . .	M, A. . .	M, A	
117b	M.	M, A. . .	M, A. . .	Dead.	
118a	H.	H, A. . .	M, A. . .	M, A	Mm.
118b	H.	H, A. . .	M, A. . .	M, A	Mm.
119a	H.	M, A. . .	M, A. . .	M, A	
119b	H.	M, A. . .	M, A. . .	M, A	
120a	M.	M, A . .	M, A. . .	M, A	
120b	M.	H, A . .	M, A. . .	Dead.	
121a	H.	H, A. . .	H, A. . .	H, A.	
121b	H.	H, A. . .	H, A. . .	H, A.	
122a	H.	H, A. . .	H, A. . .	H, A	Mm.
122b	H.	H, A. . .	H, A. . .	M, A.	Mm.
123a	H.	H, A. . .	H, A. . .	H, A.	
123b	H.	H, A. . .	H, A. . .	H, A.	
124a	H.	H, A. . .	H, A. . .	H, A.	
124b	H.	H, A. . .	H, A. . .	Dead.	
125a	H.	H, A. . .	H, A. . .	Young leaves dead.	Mm.
125b	H.	H, A. . .	H, A. . .	M. A	Mm.
126a	H.	H, A. . .	H, A. . .	M. A	Mm.
126b	H.	H, A . .	H, A. . .	Young leaves dead.	Mm.

[a] All potatoes were planted Dec. 19, 1917.

[b] H=healthy. M=mosaic. Mm=having a medium mosaic infection. A=infested with aphids.

TABLE IV.—*Effect of aphids on mosaic of potato at greenhouse, Washington, D. C., 1918–19*—Continued

Pot No.	Condition of plants on—				Performance of second generation, inspected Jan. 4, 1919.
	Jan. 28, 1918.	Mar. 1, 1918.	Mar. 19, 1918.	Apr. 6, 1918.	
127a	H.....	H, A...	H, A...	H. A	
127b	H.....	H, A...	H, A...	H. A	
128a	H.....	H, A...	H, A...	H. A	Mm.
128b	H.....	H, A..	H, A...	H. A	Mm.
129a	M.....	M, A...	M, A ..	M	Mm.
129b	M.....	M, A...	M, A...	Dead.....	Mm.
130a	H.....	H, A...	H, A...	H, A.....	
130b	H.....	H, A...	H, A...	Dead.....	
131a	H.....	H, A...	H, A...	Too mature.....	
131b	H.....	H, A...	H, A...	.....	
132a	.....	H, A...	H, A...	.....	Mm.
132b	.....	H, A...	H, A...	.....	Mm.
133a	.....	M, A...	M, A...	Dead.....	Mm.
133b	.....	H, A...	H, A ..	H	Mm.
134a	.....	M, A...	M, A...	M, A	
134b	.....	M, A...	M, A...	Dead.....	
135a	.....	M, A ..	M, A...	M, A	Mm.
135b	.....	H, A...	H, A...	H, A	Mm.
136a	.....	H, A...	H, A...	Too mature to observe.....	
136b	.....	H, A...	H, A...	A	
137a	.....	M, A...	M, A...	M.....	
137b	.....	M, A...	M, A...	Dead.....	
138a	.....	H, A...	H, A...	M.....	Mm.
138b	.....	H, A...	H, A...	M.....	Mm.
139a	.....	H, A...	H, A...	H, A.....	
139b	.....	H, A...	H, A...	H, A.....	
140a	.....	H, A...	H, A...	Too mature to observe.....	
140b	.....	H, A...	H, A...	.....	
141a	.....	H, A...	H, A...	A.....	Mm.
141b	.....	H, A...	H, A...	A.....	Mm.
142a	.....	M, A...	M, A...	M, A.....	Mm.
142b	.....	H, A...	H, A...	Dead	Mm.
143a	.....	H, A...	H, A...	M, A.....	
143b	.....	H, A...	H, A...	Dead.....	
144a	.....	H, A...	H, A...	M, A.....	
144b	.....	H, A...	H, A...	M, A.....	
145a	.....	H, A...	H, A...	.....	
145b	.....	H, A...	H, A...	.....	
146a	.....	H, A...	H, A...	M, A.....	Mm.
146b	.....	H, A...	H, A...	M, A.....	Mm.
147a	.....	M, A...	M, A...	M, A.....	
147b	.....	M, A...	M, A...	M, A.....	
148a	.....	H, A...	H, A...	H, A.....	
148b	.....	H, A...	H, A...	H, A.....	
149a	.....	H, A...	H, A...	H, A.....	
149b	.....	H, A...	H, A...	H, A.....	
150a	.....	H, A...	H, A...	H, A.....	Mm.
150b	.....	H, A...	H, A...	H, A.....	Mm.
151a	.....	H, A...	H, A...	Dead.....	Mm.
151b	.....	H, A...	M, A...	.....	Mm.

TABLE V.—*Summary of Table IV*

Condition of plants on Jan. 28, 1918.			Condition of plants on Mar. 1, 1918.			Condition of plants on Mar. 19, 1918.			Condition of plants on Apr. 6, 1918.		
Total number of plants 101a to 132a.	Number of mosaic plants.	Percentage of mosaic.	Total number of plants 101a to 151b.	Number of mosaic plants.	Percentage of mosaic.	Total number of plants 101a to 151b.	Number of mosaic plants.	Percentage of mosaic.	Total number of plants 101a to 151b.	Number of mosaic plants.	Percentage of mosaic.
62	14	22.5	102	28	27	102	40	39	102	51	50

Total number of plants grown from above progeny in second generation is 44.
Number of second generation plants showing mottling is 44.
Number of plants without mottling in first generation but mottled in second is 21.
Percentage of plants mottled in second generation but not in first is 48.

Similar experiments were performed at Washington in the winter of 1918–19. Bliss Triumph potatoes, from stock that had been rogued during the preceding season in northern Maine, were planted in two lots. One lot was kept free from aphids by fumigation while the other was subject to a heavy infestation. In the former, 11 per cent—the progeny of 2 out of 18 halved tubers—became mottled as soon as the first leaves appeared, evidently as a result of field infection. In the latter, 67 per cent, or 31 out of 46 plants—progeny of 23 halved tubers—developed mottling. The difference between 11 per cent and 67 per cent evidently was the result of aphid dispersal from neighboring mosaic plants of the same variety. The aphid-free lot was planted December 17, 1918, and was fully matured by March 22, 1919. The infested lot was planted in 8-inch pots on February 1, 1919, in a separate greenhouse but with growing conditions practically the same as those of the other. Hundreds of aphids were present upon the plants by the time they had developed to a height of 6 to 8 inches. The plants were arranged in five rows, the plants in row 1 being in contact with the originally aphid-infested plants and the other rows following in numerical order at respectively greater distances from them and therefore being less infested by the dispersing aphids. As shown in Table VI, all the plants in rows 1 and 2 showed mottling by April 4, while at that time some but not all of the plants in the other three rows were mottled.

TABLE VI.—*Relation of aphids to mosaic of potato: Continuation of experiments at greenhouse, Washington, D. C., 1918–19*

[Planted Feb. 1, 1918; observed Apr. 4, 1918]

Seed piece No.	Condition of foliage.				Number of 8-inch pots removed from aphid-infested plants.	Remarks.
	x.		y.			
521x and y		Mottled.		Mottled.	1	
522x and y		...do....		...do....	1	Mottled from beginning.
523x		...do....			1	
523y				Mottled.	2	
524x and y		Mottled.		...do....	2	
525x and y		...do....		...do....	2	
526x and y		...do....		...do....	1	
527x and y		...do....		...do....	1	
528x and y		...do....		...do....	2	Mottled from beginning.
529x and y		...do....		...do....	2	
530x and y		...do....		...do....	3	
531x and y	Healthy.		Healthy.		3	
532x		Mottled.			3	
532y			Healthy.		4	
533x and y	Healthy.			Mottled.	4	
534x and y		Mottled.	Healthy.		4	
535x and y	Healthy.		...do....		5	
536x and y		Mottled.	...do....		5	
537x and y		...do....	...do....		3	
538x and y	Healthy.			Mottled.	3	
539x and y	...do....		Healthy.		4	
540x and y	...do....		...do....		4	
541x and y	...do....			Mottled.	5	
542x and y		Mottled.		...do....	5	Mottled from beginning.
543x and y		...do....		...do....	5	Do.

Total number of plants is 46.
Number of plants showing mottling Apr. 4, 1919, is 31.
Percentage of plants showing mottling Apr. 4, 1919, is 67.

Somewhat similar evidence was secured during the same winter at Orono, Me. Some Green Mountain potatoes were used that had been grown in a rogued plot in northern Maine during the season of 1917 and had been kept for about a year in cold storage. One lot of 10 tubers was planted immediately and 2 of them, or 20 per cent, produced plants that were mottled when very young, evidently through field infection. Another lot of 30 tubers was stored in a cellar for a few weeks and then was found to have produced sprouts that had become lightly infested with green peach or spinach aphids. These aphids apparently had dispersed from a neighboring heavily infested lot of sprouted tubers that had come from a purely mosaic stock and that later produced mosaic plants. The number of insects on a tuber varied from 0 to 30, and there were few skins and but little honey-dew deposit present. The infested lot was fumigated and planted. Five tubers, or 17 per cent, produced plants that became mottled when very small, in 25 to 30 days after planting, evidently the result of field infection. In addition to these, 6 other tubers, or 20 per cent, produced both mottled and healthy shoots. This increase can be explained only by the infestation of the sprouts by the aphids from the diseased tubers. This explanation receives support from the observation that the mottled shoots of the 6 partly diseased

tubers showed the symptoms later, averaging 44 days after planting, and that they usually came from eyes of the bud end and therefore were probably the first to become exposed to aphid attack.

GREENHOUSE EXPERIMENTS WITH INSECT CAGES

As has been indicated already, plants that appear healthy may produce tubers that develop mottled plants. In studies with potato mosaic, therefore, it is very desirable to grow a second generation if the effects of a given treatment are to be fully disclosed. Under greenhouse conditions, especially in Maine, it is necessary to furnish treated plants with as much light as possible if a satisfactory crop of tubers is to be secured. This makes it appear better, in experiments involving the artificial introduction of aphids, to remove as soon as possible any cages that were used. This can be done without compromising the results of the experiments if frequent inspection and fumigation are employed to keep insects reduced to negligible numbers.

During the winter of 1918–19 an experiment was performed in the greenhouse at Orono, Me., with Green Mountain potatoes that had been grown in a rogued plot in northern Maine during the season of 1917 and had been kept for about a year in cold storage. Fifteen tubers were planted, of which 3, or 20 per cent, produced plants which showed mosaic symptoms when only a few inches tall. The same rogued stock when planted in the field in 1918 had shown mottling in 11 per cent of the hills. The other 12 tubers, each being divided into 2, 4, or 5 sets, furnished 53 plants. Twenty-one plants, 1 or 2 from each tuber, were kept as untreated controls throughout the experiment and remained healthy. Eighteen plants, 1 or 2 from each tuber, were fed upon by aphids introduced from mosaic potato plants; and 13 of them, or 72 per cent, eventually developed typical mosaic symptoms. Five plants, from 5 tubers, were fed upon by aphids introduced from a healthy potato plant; 8 plants, from 8 tubers, were infested by aphids from radish plants; but all of these remained healthy.

In this experiment spinach aphids[1] were used and were never found, during frequent inspections, to be parasitized by other insects or by fungi or to be mixed with predatory enemies or with individuals of another aphid species. They were secured from two colonies, one on a mosaic-diseased potato plant and the other on an apparently mosaic-free one. Stock from the former was kept on mosaic-diseased potato plants and that from the latter on healthy ones or on radish plants until ready for use. The aphids were transferred to the plants of the experiment by methods that seemed favorable to the transmitting of mosaic: (1) By laying one or two leaves, bearing feeding aphids, upon the plant so that

[1] Determinations were made by Dr. Edith M. Patch, Entomologist of the Maine Agricultural Experiment Station, who informs one of the writers that this species frequently is abundant upon potato plants in Aroostook County and other parts of Maine.

the insects could crawl most easily to the new host; (2) by introducing aphids when the new host was young, 3 to 13 inches tall; and (3) by introducing a rather large number, 40 to 220 by estimate. Cylindrical cages consisting of coarse wire screening covered with fine cloth gauze (*5, Pl. 6 B*) were used to confine the aphids to the individual treated plants. These effectually served their purpose. For the three treatments with aphids—from mosaic potato, healthy potato, and radish—the height of the tallest shoot of the new host when the aphids were introduced was on the average 6, 5, and 7 inches, respectively. The number of aphids introduced was on the average respectively 130, 80, and 120, while the average number of days the insects remained was respectively 7, 14, and 9. After feeding on the new host for a week or longer, the aphids were killed by nicotine fumigation intense enough to cause the margins and tips of some leaflets to become yellow and later to die. This yellowing occurred on both the aphid-infested and aphid-free plants and was in no way similar to mosaic mottling. It did not occur on the leaves which were the first to show mosaic symptoms. Frequent cyanid and nicotine fumigation of the uncaged plants was practiced. No white flies (*Aleyrodes vaporariorum* Westw.) and very few dispersed aphids were found at any time in the room occupied by the plants included in this experiment. No other species of aphids was found in the greenhouse. Thrips fed somewhat upon all the plants, both those within cages and those uninclosed.

The aphids were introduced in December and January. Symptoms of mosaic were first seen in 18 to 31 days and then consisted of the mottling characteristic of "slight" mosaic, but the mottling soon became more pronounced and sometimes was accompanied by considerable wrinkling. The average number of days that elapsed between the introduction of the aphids and the time when mosaic symptoms were first ascertained was 26. It might have been shorter if the plants had been examined daily instead of semiweekly. The average height of the tallest shoot at the time when the symptoms were first ascertained was 20 inches. The symptoms appeared first in the one, two, or three topmost leaves of an affected shoot, which, if already formed, were still very small at the time the aphids were feeding on the plant.

The fact that a large percentage of the plants treated with aphids from mosaic potatoes showed mosaic while the others, either untreated or treated with nonvirulent aphids, all remained healthy, can be attributed only to aphid transmission. As pointed out before, the group of plants that showed mosaic came from the same tubers as the healthy controls. Moreover, special precautions were followed because of the previous tendency to regard mosaic as a physiological disease and therefore to neglect some operations normally followed in pathological work. All the plants were grown in the same greenhouse room and were arranged so that those with each type of treatment were distributed

over the bench, all four treatment groups thus being mixed and apparently exposed to similar conditions of light, temperature, and humidity. Each plant had enough space so that it was not in contact with any other. Soil fertilization and watering were similar for all plants. There was as much variation in the amount and type of soil used for the plants that showed mosaic symptoms as for the others. The untreated controls came from neither eye-end sets nor stem-end sets. Each tuber was cut with a flamed knife, and the seed pieces were planted in steam-sterilized soil. Finally, the objection that the method used for introducing aphids brought in the factor of contact with diseased leaves, is met by the results of 14 checks in another room of the greenhouse. These 14 tubers from the same stock produced tuber hills, each of which remained entirely healthy for 38 days after a mosaic leaf or shoot had been placed upon it when it was 8 inches high.

In connection with the experiment reported in Table VI another aphid experiment was conducted at Washington, D. C., in the winter of 1918–19, but with a Green Mountain lot and with insect cages employed. From this stock for the last three seasons the mosaic plants had been eliminated, so that but 13 per cent of the plants developed mosaic as soon as new leaves were formed. In this experiment, plants from 5 different tubers were used. Each of these tubers was halved, making 10 sets. Plants from 5 different sets, designated 474x, 481x, 486x, 470x, and 478x, were kept in the greenhouse without a cage; and plants from the corresponding 5 different sets, designated as 474y, 481y, 486y, 470y, and 478y, were placed in two cages which were kept in the same greenhouse with the uncaged plants. Three of these plants, 474y, 481y, and 486y, when from 3 to 6 inches tall were placed in one cage, while the two remaining plants, 470y and 478y, were placed in another cage. On February 26, 1919, a few hundred aphids taken from healthy Green Mountain plants were transferred to each of plants designated 470y and 478y, and similar transfers were made on March 1 and on March 15. The aphids were brushed upon cardboard with a camel's-hair brush and then transferred to the plants. Before the transfers were made on March 15 the plants were fumigated. Upon 474y, 481y, and 486y aphids from mosaic plants were transferred in a similar manner, but with a different brush and cardboard. Here also three distinct transfers were made on February 26, March 1, and March 15. The plants were fumigated on March 15 and the third transfer made. At this time a few hundred aphids were placed upon each of the plants. This last set of aphids was allowed to feed on the plants until March 22, when the lower half of each stalk had become defoliated. Then another tobacco fumigation was applied, and the cages were removed from the plants. At this time a few of the newly formed leaves showed distinct mottling. On April 2 newly formed leaves on all 7 stalks representing the 3 different plants were distinctly mottled (Pl. 30, A). At this time

the 3 plants which had developed from the other half tubers and had been kept free from aphids were free from mottling. On examination of the control plants 470y and 478y as well as 470x and 478x on April 4, no mottling whatever was found (Pl. 30, B).

In January, 1919, some Green Mountain potatoes were secured at Orono, Me., supposedly from a field that had been found free from mosaic the previous season. Seventeen tubers were divided each into 6 seed pieces. Eight tuber groups of 6 plants each developed mottling when very small, and the other 9 did not. The 6 plants from each tuber were subjected to 6 different treatments: One plant was kept as an uncaged control; another was a control, caged until the plant was over 2 feet tall; the third was grown intertwined with a mosaic potato plant from a separate pot; the fourth was fed upon for a week by wingless green peach aphids from a mosaic potato plant, an average number of about 130 being introduced on a piece of gauze when the plant was 3 inches high; the fifth received the same treatment as the fourth except that the average number of insects was about 170 and that they were introduced on leaves which were impaled upon a sterile stick thrust into the soil in such a way that there was no contact between the introduced leaves and the plant or soil (see Pl. 26, B); on the sixth plant when 1 inch high there were placed 20 winged aphids secured from a mosaic plant with a camel's-hair brush and introduced within a small open bottle.

All of the 18 controls remained healthy. Of the 9 plants with aphids introduced on leaves, as described above, 8, or 89 per cent, became mottled in 20 to 31 days—averaging 25 days—or when the plants had become 14 to 29 inches high—averaging 25 inches. One of these plants, together with an untreated plant from the same tuber, is shown in Plate 29, B, and corresponding leaves from these two plants are shown in Plate 27, C. Of the 9 plants with aphids introduced on gauze, 2, or 22 per cent, became mottled in 20 to 26 days, when the plants were 22 to 29 inches high. Of the 9 plants with winged aphids introduced, 1, or 11 per cent, showed signs of mosaic in 27 days, when 17 inches high. Of the 9 plants kept in contact with mosaic plants, all remained healthy but 1. This 1 was brought into contact with the diseased plant on March 7, was found to be free from aphids on March 31, was fumigated on April 7 because of the presence of several aphids, and showed signs of mosaic on April 17, when 35 inches high. This plant became diseased apparently either because of transmission by very few aphids after March 31 or because of contact. The latter cause seems more probable, but would make this the only case of contact transmission known at present to the writers.

This experiment seems to have demonstrated that aphids can transmit mosaic, even better than the first experiment conducted in this greenhouse (p. 262–264). The same precautions were used in this experiment,

and in addition each tuber was split lengthwise so that each seed piece included eyes from the bud-end and the stem-end. Also, no mosaic leaves were put in contact with the plants when aphids were introduced, and all cages that had been used previously were steam sterilized.

PHYSIOLOGICAL STUDIES

Some work on the chemical differences between the healthy and diseased potato plants was carried out in connection with this investigation. These experiments included a determination of the reducing and total sugars and starch content in the healthy and diseased leaves of potato plants grown under the same environmental conditions. In this work the potato leaves were picked off the stems and weighed. They were dried in a hot oven to constant weight and extracted with alcohol in a Soxhlet extractor. The sugars were determined in the extract, and the starch in the residue. The results of these determinations are given in Table VII. Data are given in this table as to the location of the plants from which the samples were taken, whether caged or in the open, the time of sampling, and whether the day was bright or cloudy. In the other columns of the table are given the results of the sugar and starch determinations. The determinations compared in the table are from plants grown under as nearly the same conditions as possible.

TABLE VII.—*Sugars and starch in healthy and mosaic Green Mountain potato foliage*

Time, treatment of plants, and weather conditions when sampled.	Percentage of sugars as dextrose on basis of dry weight.						Percentage of starch on basis of dry weight.	
	Reducing sugar.		Nonreducing sugar.		Total sugar.			
	Healthy.	Mosaic.	Healthy.	Mosaic.	Healthy.	Mosaic.	Healthy.	Mosaic.
11 a. m. Plants caged. Cloudy day.....	1.6	2.8	3.5	3.7	5.1	6.5	25	17
11.30 a. m. Plants in open. Bright day.....	2.3	2.4	5.5	5.5	7.8	7.9	28	19
9.30 a. m. Plants in open. Bright day.....	2.5	3.0	7.0	5.1	9.5	8.1	21 18	16 19
2.10 p. m. Plants caged. Bright day.....	1.0	1.6	1.1	3.3	2.1	4.9	23	15
4.15 p. m. Plants in open. Rainy day.....	2.3	4.4	4.2	4.3	6.5	8.7	17 13	15 15
Averages.....	1.94	2.84	4.26	4.38	6.20	7.22	20.7	16.6

From the results shown in Table VII it appears that mosaic plants have a higher sugar content than the healthy plants grown under the same conditions. This is true of both reducing and nonreducing sugars, though the differences in the latter are not so marked. There is an average of about 1 per cent more total sugar in the mosaic plants than in the healthy. With starch this relation is reversed, healthy plants having an average of about 4 per cent more starch than those affected with mosaic. It is, of course, to be remembered that the investigations

in this paper are preliminary in character. It is hardly possible to draw conclusions from so limited an amount of data, though the facts seem worth recording.

METHODS OF CHECKING NATURAL TRANSMISSION

The experimental results previously described in this paper suggest at least one way in which transmission of potato mosaic may occur in the field—namely, by aphids. Both species of aphids that were experimented with are commonly found on potatoes, including those in Aroostook County, Me. In 1918, a year in which aphids were unusually abundant upon potatoes in northern Maine, they began to appear upon the plants about the middle of July. Since in the experiments mottling did not appear after the plants had finished elongating and had produced blossoms, it is quite probable that aphid transmission in the field occurs too late for the effects to be shown during the same season. The possibility of this was demonstrated in one experiment (p. 262–264) in which after aphid transmission some plants remained unmottled but produced progeny that showed disease the next season. Before evidence had accumulated regarding insect transmission, control of the disease was attempted by means of hill selection and roguing. The results of such attempts, together with notes made at the same time on yields, will now be discussed.

HILL SELECTION

A number of hill selections were made in 1916 and 1917 in northern Maine in order to ascertain more especially the progress of mosaic from one season to another upon the same strain and stock. Plants in three different stages of the disease as well as healthy checks were included in these selections. The term "slight stage" was used when the plants had just begun to show a few mottled spots on the leaves though the foliage otherwise appeared like that of healthy plants. "Medium stage" was used when the leaves had apparently just begun to become slightly corrugated, had six or more mottled areas, and had begun to show slight dwarfing. "Bad stage" indicated that the leaves were mottled, corrugated, and decidedly dwarfed. The results of the observations on the behavior of the foliage are presented in Table VIII.

TABLE VIII.—*Hill selection: Effect of mosaic of potato on vines in laboratory plots, Presque Isle, Me., 1918*

Variety.	Planted for—	No. of strain.	No. of parent hill.	No. of tubers planted.	No. of seed pieces.	No. of parent hills producing all healthy plants.	No. of parent hills producing healthy and mottled plants.	No. of parent hills producing all mottled plants.	No. of tubers producing mottled and nonmottled plants.	No. of mosaic hills.	No. of healthy hills.	No. of plants in each stage of mosaic.		
												Slight.	Medium.	Bad.
Bliss Triumph	Control to mosaic	1	8	31	122	1	7	0	1	53	69	3	40	10
Green Mountain	do	1	6	30	119	4	2	0	2	24	95	....	24	....
Do	do	1	11	39	284	3	3	5	1	147	137	1	146	....
Do	do	1	5	14	82	0	1	4	0	70	12	....	70	12
Do	do	1	3	8	56	0	0	3	0	....	....	....	44	12
Irish Cobbler	do	1	11	31	116	5	3	3	1	34	82	....	22	12
Do	do	1	9	27	117	6	3	0	4	22	95	....	....	....
Green Mountain	Slight	1	2	2	7	0	0	7	0	7	0	....	7	....
Do	do	1	4	15	74	0	....	4	0	74	....	....	74	....
Do	Very slight	1	5	12	84	0	1	4	2	6	78	66	12	....
Do	do	1	8	24	151	3	2	3	0	70	87	12	54	4
Do	Slight	1	4	14	82	0	0	4	0	....	....	76	6	....
Bliss Triumph	do	1	9	20	79	0	0	9	0	79	....	27	52	....
Green Mountain	Medium	1	4	8	32	0	0	4	0	32	....	8	16	8
Do	do	1	2	5	30	0	0	2	0	20	....	....	20	....
Do	do	1	5	11	63	0	0	5	....	63	....	....	55	8
Do	do	1	6	14	75	0	0	6	....	75	....	3	72	....
Do	do	1	5	10	39	....	....	....	....	39	....	....	39	....
Do	do	1	5	10	40	....	....	....	....	40	....	....	28	12
Do	do	1	3	6	36	....	....	....	....	....	....	....	36	....
Bliss Triumph	Bad	1	10	20	80	....	....	....	....	....	....	....	16	64
Green Mountain	do	1	3	6	24	....	....	....	....	....	....	....	8	16
Do	do	1	2	4	15	....	....	....	....	....	....	....	4	12
Do	do	1	4	8	32	....	....	....	....	32	....	....	28	4
Do	do	1	2	4	16	....	....	....	....	16	....	....	....	16
Do	do	1	3	6	24	....	....	....	....	24	....	....	16	8
Do	do	1	8	16	32	....	....	....	....	32	....	....	....	32
White Bliss	Medium	1	10	20	39	....	....	....	....	39	....	....	39	....
Bliss Triumph	do	1	10	20	40	....	....	....	....	40	....	....	32	8
Irish Cobbler	Dwarf	1	11	22	76	....	....	....	....	76	....	....	76	....
Do	do	1	2	4	12	....	....	....	....	12	....	....	12	....
Green Mountain	Medium	1	3	6	24	....	....	....	....	24	....	....	24	....
Do	do	1	11	17	32	....	....	....	....	32	....	....	32	....
Do	Slight	1	4	7	42	....	....	....	....	42	....	....	42	....
Irish Cobbler	Control to streak and mosaic.	1	21	47	202	....	....	....	5	24	178	....	14	10
Do	do	1	3	7	42	....	....	....	2	....	30	....	5	7

From these data it is apparent that progeny from plants seeming to be healthy in one season may develop both healthy and diseased stock the following season. It will be noted further that the mottling on this control stock the following season may develop to such a degree that it falls under all three stages, slight, medium and bad, and does not necessarily begin with a slight stage as one might expect. Furthermore, it is shown that mottled and nonmottled plants may develop from the same hill and even from a single tuber, similar observations having been recorded by F. C. Stewart (*10*).

The observations regarding degree of mottling and dwarfing in the control stock also obtained in the slight, medium and bad stages. In none of these stages did the stock necessarily run true to the stage for which it was selected. It will be noted also that mottled foliage developed wherever the progeny came from plants showing the slight, medium, or bad stage the previous season. In but two strains where the foliage

appeared to be very slightly mottled but questionable for mosaic in 1917 were mottled and nonmottled plants noted in 1918. Observations upon the foliage symptoms were made at three different times during July and August, the first observations being made when the vines were from 2 to 6 inches tall and the last just before the vines began to die.

In connection with the observations on the behavior of the vines of hill-selected stock, studies were made also on the effect of mosaic on yield. The hills were selected from some of the same stock on which notes upon the performance of the foliage were taken, and hence the stages of the disease indicated here answer the same description as those presented in connection with the notes upon the behavior of the foliage as indicated in Table VIII. Table IX gives the effect on yield of these hill selections.

TABLE IX.—*Hill selections: Effect of mosaic of potato on yield in laboratory plots, Presque Isle, Me., 1917–18*

Plot No.	Condition of stock in 1917 and 1918.	Hill unit.	Number of hills.	Total yield.		Average yield per hill.		Yield of parent hill in 1917.		Yield per hill in 1918 compared with that of 1917.	
										Increase.	Decrease.
				Lb.	*Oz.*	*Lb.*	*Oz.*	*Lb.*	*Oz.*	*Oz.*	*Oz.*
4	Healthy	4A	8	15	14	1	15	1	5	10	.....
4	.....do	4C	8	15	0	1	14	1	13	1	.....
4	.....do	4D	6	11	6	1	14	1	9	5	.....
4	.....do	4E	3	6	14	2	5	1	9	12	.....
4	.....do	4F	2	3	12	1	14	1	14	.....	.....
5	.....do	5A	2	3	8	1	12	1	9	3	.....
5	.....do	5B	3	5	12	1	15	1	6	9	.....
5	.....do	5C	4	7	0	1	12	1	6	6	.....
5	.....do	5D	2	3	12	1	14	1	4	10	.....
5	.....do	5E	11	22	2	2	2/11	1	14	2	.....
5	.....do	5F	14	28	13	2	13/14	1	12	5	.....
16	Slight	16B	4	6	8	1	10	1	0	10	.....
16	.....do	16H	2	2	8	1	4	1	0	4	.....
16	.....do	16I	6	7	4	1	3		13	6	.....
25	Bad	25A	4		15		4		6	.....	2
25	.....do	25E	4	2	1		8		7	1	.....
25	.....do	25F	4	1	4		5		8	.....	3
25	.....do	25H	4	1	15		7		8	.....	1
25	.....do	25J	4	1	4		5		7	.....	2
36	Medium	36A	4	7	2	1	13	1	3	10	.....
36	.....do	36B	4	8	2	2	1/2	1	6	11	.....
36	.....do	36C	4	5	10	1	7	1	0	7	.....
37	.....do	37A	4	6	14	1	12	2	8	.....	12
37	.....do	37B	4	4	8	1	2	1	10	.....	8
37	.....do	37C	4	5	14	1	8	1	8	.....	.....
37	.....do	37D	4	6	4	1	9	1	12	.....	3
37	.....do	37E	4	5	4	1	5	1	15	.....	10
37	.....do	37F	2	3	0	1	8	1	11	.....	3
37	.....do	37G	1	1	12	1	12	1	6	6	.....
37	.....do	37H	2	2	6	1	3	1	6	.....	3
37	.....do	37I	3	2	8		13	1	1	.....	4
37	.....do	37J	2	2	6	1	6	1	9	.....	3
37	.....do	37K	2	2	14	1	7	1	4	3	.....

In this table the yield per hill in the season of 1918 is compared with that of 1917. From control plants and those slightly mottled there was a slight increase in yield, whereas from plants showing medium and bad stages of mottling there was in some an increase but in a larger number a decrease. Although it will be necessary to study the performance of such stock for a number of seasons before final conclusions upon the effect of mosaic on the yield can be submitted, nevertheless it is clear that mosaic hills can be depended upon to produce diseased progeny, while apparently healthy hills can not be depended upon to produce healthy progeny. Consequently hill selection is an unsatisfactory method of control, at least when practiced in a field that contains a considerable percentage of affected hills.

ROGUING

Additional observations on the effect of mosaic of potato on yield were made in connection with the experiments on roguing. In these experiments the stock was not hill-selected but was harvested in bulk after the affected hills were removed from the plots during the growing season. The results of these observations as well as of those on the behavior of the vines are indicated in Table X.

From these data a reduction in yield of from 23 to 30 per cent is apparent where progeny from wholly diseased lots is compared with the progeny from lots of the same strain and variety but with a low percentage of mottled plants. Furthermore, the mottled plants were reduced from 45 per cent in 1917 to 13 per cent in 1918 as the result of but one thorough roguing in 1917, when the plants were about 12 inches tall. However, in order to note how much the percentage of mottled plants can be reduced by roguing it will be necessary to study the effect of this procedure on the same stock and strain for several seasons and under as nearly uniform conditions as possible.

By reducing the number of diseased plants in the seed stock the effect of the aphids in spreading the disease is apparently considerably reduced. It is quite evident that such roguing must be carried on with the greatest care and by persons who are thoroughly acquainted with the symptoms of the disease. Even though practically all diseased plants can be eliminated with a single roguing in one season, the work can be done more efficiently with two or three roguings, beginning when the plants are from 6 to 10 inches tall. With this method it is advisable to begin with a stock which runs relatively low in the number of affected plants.

Whether it is possible entirely to eliminate mosaic by roguing has not been proved. From the results of the study of aphid transmission here reported it is evident that attempts to eliminate mosaic by roguing should be made on an isolated seed plot removed from aphid-infested fields. In addition, insects of all kinds should be kept off the seed plot by adequate spraying. Naturally the same precautions should be taken if one wishes to prevent transmission of the disease to seed plots or fields planted with mosaic-free seed tubers.

TABLE X.—*Effect of roguing on mosaic of potato at Aroostook Farm, Presque Isle, Me., 1918*

Plot No.	Selected for—	Variety.	Treatment in 1917.	Number of hills.	Area	Total yield.	Yield per acre.	Yield per acre.	Yield per hill.	Decrease in yield per acre.	Mosaic on vines.	Remarks.
					Sq. ft.	*Pounds.*	*Pounds.*	*Barrels.*	*Pounds.*	*Per cent.*	*Per cent.*	
1	Control..	Green Mountain...	Rogued 3 times.....	2,209	8,880	3,000	14,716	89.12	1.35	.....	11.44	
8	Mosaic..	.....do.....	Rogued all nonmottled plants.	2,916	10,416	2,716	11,312	68.55	.93	23.12 less than plot 1.	100.00	Same strain as plot 1.
2	Control..	Bliss Triumph....	Rogued 3 times.....	2,201	9,480	2,697	12,392	74.49	1.22	.....	15.33	
7	Mosaic..	.....do.....	Rogued all nonmottled plants.	2,095	8,608	1,734	9,681	52.61	.82	29.94 less than plot 2.	100.00	Same strain as plot 2.
3	Control..	Green Mountain...	Rogued once. Seed tuber 2 ounces +.	2,352	10,008	3,194	13,901	84.24	1.31	.....	13.00	
4	Mosaic..	.....do.....	No roguing. Seed tuber 2 ounces +.	2,924	10,008	3,214	13,988	84.77	1.09	.....	44.88	Same strain as plot 3.
5	...do.....	.....do.....	No roguing. Large and small seed tubers.	2,270	8,201	2,704	14,362	87.04	1.10	.....	45.52	Do.
6	...do.....	.....do.....	No roguing.....	864	3,689	1,112	13,130	79.53	1.28	.....	10.16	Good commercial strain.

SUMMARY

(1) Mosaic of the Irish potato has become well distributed over the United States.

(2) It has a decidedly detrimental effect upon yield.

(3) It produces characteristic symptoms upon the aerial parts of the plant, especially on the foliage. These symptoms may be modified or obscured by differences in environment or variety.

(4) Tubers of diseased plants carry the disease.

(5) Grafting a healthy scion upon a diseased stock, or a diseased scion upon a healthy stock, may result in the development of the disease by the originally healthy scion or stock.

(6) Mosaic may be transmitted by transferring juice from a diseased plant to a healthy plant.

(7) At least two species of aphids can transmit potato mosaic, whether the aphids are transferred artificially or disperse naturally.

(8) Mosaic apparently tends to increase the sugar content of the leaves and to reduce their starch content.

(9) Hill selection has not proved successful for maintaining healthy stock when practiced in fields having a considerable number of mosaic plants.

(10) Roguing or eliminating mosaic plants before aphids become abundant is indicated indirectly by certain experimental evidence here presented as being helpful and also has been found actually efficient for checking the spread of the disease. It appears also that isolation of the rogued seed plot is very desirable.

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PLATE A

Foliage of Irish potato, Green Mountain variety. Note distinct mottling and slightly lighter color of diseased leaves on plant at left. Single dark green leaflet from healthy plant at right. Presque Isle, Me., 1915.

A. H. GRAHAM E.O. WASHINGTON, D.C.

A.B.BRAHAM CO.WASHINGTON, D.C.

PLATE B

Foliage of potato, Bliss Triumph variety. Note decided crinkling of leaf parenchyma on diseased leaf at left. More severely affected than diseased leaf on Plate A. Healthy leaf of same variety at right. Greenhouse, Washington, D. C., 1919.

PLATE 25

Leaf of Irish potato, Green Mountain variety, infected with mosaic. Medium stage of disease. Note mottling and crinkling of laminar parenchyma. Specimen taken from field, Caribou, Me., 1914.

B
A

PLATE 26

A.—Healthy scion grafted upon diseased stock. Younger leaves on scion show typical mottling. Greenhouse, Washington, D. C., 1916.

B.—An illustration of a method used for introducing aphids. This method resulted in 89 per cent of infection before the plants matured. In practice the insect cage was left in place while the aphid-bearing leaves on the stick were introduced. Greenhouse, Orono, Me., April, 1919.

PLATE 27

A.— Leaves from graft shown in B, of this plate: At right, from healthy parent of scion; at left, from mosaic stock; in center, from mosaic scion.

B.—At left, healthy scion grafted to diseased stock, Green Mountain variety; at right, two mosaic shoots of stock. Grafted July 6, 1918. Scion decidedly mottled August 17, 1918. In field, Presque Isle, Me., 1918.

C.—Leaves from corresponding parts of the plants shown in Plate 29, B. These leaves were near the tops of the plants and matured long after all aphids had been removed. The characteristic mottling was obscured by the use of reflected light, but the contrast in the evenness of the leaf surfaces is evident. Greenhouse, Orono, Me., 1919.

A
B
C

B
A

PLATE 28

A.—491x, inoculated artificially with unfiltered juices from mosaic plant February 22 to March 22, 1919. Note mottled leaves on the two new shoots at apex of plant. Green Mountain variety. 491y, control, untreated plant from half of same tuber as 491x. Greenhouse, Washington, D. C., 1919.

B.—473y, inoculated in same way as 491x, but with juices from healthy plant. 485y, also inoculated with juices from healthy plant. Greenhouse, Washington, D. C.

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PLATE 29

A.—Mosaic of potato transmitted by aphids. 142a, infected plant, Green Mountain variety. Plant developed beside a badly mosaic plant, thus allowing free infection by the aphids. Upper leaves distinctly mottled and crinkled. Lower leaves without mottling. 142b, healthy plant from same tuber as 142a. Greenhouse, Washington, D. C., March 14, 1918.

B.—Two plants from the same tuber treated alike, except that about 200 aphids were introduced upon one when it was 2 inches high. Photographed 46 days after the introduction of aphids and 26 days after the first signs of mosaic were shown. Greenhouse, Orono, Me., April, 1919.

B
A

A
B

PLATE 30

A.—Inoculated by means of artificial transfers of aphids from diseased plants. Green Mountain variety. Transfers made February 26, March 1, and March 15, 1919. Distinct mottling and crinkling of younger leaves noted April 1, 1919. Greenhouse, Washington, D. C.

B.—Plants inoculated in same way as those in A of this plate, but with aphids taken from healthy plants. No mosaic April 1, 1918. Greenhouse, Washington, D. C.

TEMPERATURE IN RELATION TO QUALITY OF SWEET-CORN

By Nan. E. Stevens, *Pathologist, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*, and C. H. Higgins, *Instructor in Chemistry, Bates College, Lewiston, Me.*[1]

INTRODUCTION

The temperature at which green sweetcorn (*Zea mays*) is held after picking has an important relation to its quality. Certain features of this relation are discussed in the present paper. That sweetcorn canned near the northern limit of its cultivation is sweeter and its general quality superior to that canned farther south seems to be generally accepted (*9, p. 249; 10, p. 36*).[2] The correctness of this belief is attested by the fact that it has been customary for many corn growers in Maryland, for example, to purchase northern-grown seed in the belief that a sweeter corn would thus be obtained (*10, p. 31*), and by the reputation of "Maine sweetcorn."

That any difference in the quality of the canned corn is not due to a difference in the sugar content of the corn when it is picked seems fully proved by the investigations of Straughn and Church (*14*). These investigators determined the sugar content of freshly picked corn of the same variety at a series of stations located in Florida, South Carolina, New Jersey, Connecticut, and Maine during the four years from 1905 to 1908. In contrast to the condition found in sugar beets, this work failed to show any direct relation between the latitude in which the corn was grown and the sugar content. Corn grown in South Carolina showed the highest percentage of sugar, that grown in Connecticut the lowest, that from Maryland and Maine (Crosby variety) intermediate and about equal (*14, p. 62*).[3]

The writers believe that the advantage of northern-packed corn lies, at least in part, in the lower temperatures at which it is handled, and the present paper aims to present the following salient points in this connection: (1) That sweetcorn deteriorates very rapidly after it is picked, (2) that the rate of this deterioration depends upon temperature, and (3) that the differences in climatic temperatures, and consequently in the

[1] The work on which the present paper is based was done while the writers were investigating the diseases of sweetcorn in Maine, through the courtesy and at the expense of the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 283–284.

[3] The curves published by Straughn and Church (p. 59–60) are somewhat misleading, since, as explained in the text, results from analyses of both Crosby and Stowell varieties are included for Maryland and only the Crosby variety, having a higher sugar content than the Stowell, was grown in Maine.

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temperatures at which the corn remains after picking, are sufficient to cause marked differences in the rate of deterioration. Whether the main thesis be accepted or not, the data presented are sufficient to indicate that a close relation exists between the quality of sweetcorn and the temperature at which it is handled.

LOSS IN SUGAR AFTER PICKING

That green corn deteriorates rapidly after picking is a matter of common observation, and that an important factor in this deterioration is the loss in sugar has been pointed out by Straughn, Appleman, and others. Straughn, working with Stowell's Evergreen in Maryland, reports (*13, p. 69*) that in freshly pulled samples 4.59 to 4.74 per cent total sugars were found. On standing 24 hours at room temperature, unhusked, about one-third of the sugars disappeared; after this the loss continued until the sugars reached 1.80 per cent. More recently, Appleman and Arthur (*2, Table III*), working with the same variety stored at accurately controlled temperatures, report that at 20° C. more than 25 per cent of the total sugar was lost during the first 24 hours after picking, and that at 30° C. more than 50 per cent of the total sugar was lost in the same period. Analyses of Golden Bantam corn made at Lewiston, Me., during 1918 showed rapid loss in sugars in stored corn. The ears were split lengthwise and a sample from one half analyzed immediately, while the other half was stored.

TABLE I.—*Total sugars in Golden Bantam corn in edible condition, calculated as percentage of invert sugar on original wet weight, Lewiston, Me.*[1]

Ear No.	Percentage of sugars in fresh half.	Percentage of sugars in stored half.	Approximate number of hours stored at 20° C.
2	5.36	2.39	24
6	4.40	2.79	20
9	5.84	2.72	48
10	5.94	2.46	48

[1] The method used was essentially that of Bryan, Given, and Straughn as modified by Hasselbring and Hawkins. The total sugars were calculated as invert sugar by the methods of Munson and Walker. BRYAN, A. H., GIVEN, A., and STRAUGHN, M. N. EXTRACTION OF GRAINS AND CATTLE FOODS FOR THE DETERMINATION OF SUGARS ... U. S. Dept. Agr. Bur. Chem. Circ. 71, 14 p., 1911; HASSELBRING, Heinrich, and HAWKINS, Lon A. PHYSIOLOGICAL CHANGES IN SWEET POTATOES DURING STORAGE. *In* Jour. Agr. Research, v. 3, no. 4, p. 335, 1915; WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS ... U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 241, 1908.

RELATION OF TEMPERATURE TO RATE OF SUGAR LOSS

The recently published careful researches of Appleman and Arthur (*2*) explain the earlier and somewhat conflicting statements of Straughn, Church, and Wiley (*13, 14*) and leave no doubt that the rate of loss of sugar in stored sweetcorn is directly dependent on temperature. Apple-

man and Arthur summarize two years' work on Stowell's Evergreen stored at seven carefully controlled temperatures, namely, 0°, 5°, 10°, 15°, 20°, 30°, and 40° C., as follows:

> In general, it may be stated that up to 30° C. the rate of sugar loss in green corn is doubled for every increase of 10°. This applies to both total sugars and sucrose. It should be noted, however, that between 0° and 10° the temperature coefficient for sucrose is considerably greater than 2.

Before the conclusions of Appleman and Arthur were available, the writers made a few tests to determine whether temperature influenced the rate of sugar loss in sweetcorn. Their results agree closely with his, but since the work was done on another variety grown at a considerable distance the data secured may still be of sufficient interest to warrant publication. Freshly picked ears of Early Bantam corn in edible condition were split lengthwise. One half was placed in a small refrigerator which maintained a temperature of approximately 10° C. and the other half placed in a box at room temperature, about 20°. Determinations made at the end of 26 to 30 hours showed uniformly a lower sugar content in the half kept at the higher temperature. In most cases the ears were too small to make three satisfactory samples, so no data are available for the original sugar content of the ears used. The freshly picked ears listed in Table I were, however, of the same variety, grown in the same plot, and picked at the same stage of maturity as those shown in Table II; and if the ears used in the keeping test had about the same average sugar content as those in Table I (5.38 per cent of wet weight) then the halves kept at 20° lost, during the first day after picking, more than twice as much sugar (3.36 per cent) as the halves kept at 10° (1.41 per cent).

TABLE II.—*Total sugars in Golden Bantam corn in edible condition, calculated as percentage of invert sugar on original wet weight, Lewiston, Me., September, 1918*

Ear No.	Percentage of sugar remaining in half stored at 20° C.	Percentage of sugar ramaining in half stored at 10° C.
11	2.43	4.06
12	1.90	3.14
13	2.28	5.54
14	1.90	3.71
16	1.86	3.18
17	1.78	4.21
Average	2.02	3.97

Further evidence that the rate of vital activities of green sweetcorn varies with temperature is afforded by tests of the rate of respiration. The curves of respiratory intensity of sweetcorn during storage published by Appleman (*1, p. 207*) show that the rate of respiration is very high during the first day after the corn is pulled from the stalk but falls off rapidly with storage. They clearly show also that throughout a storage

period of nine days respiration continued more rapidly at 30° C. than at 25°. In storage tests made by the present writers during 1918, 48 ears of freshly picked green corn were placed in an air-tight can which had a capacity of 46.6 liters. The corn displaced somewhat more than 20 liters, leaving about 26 liters of air. The can was then sealed, and 100-cc. samples of the air were withdrawn at intervals through a stopcock and analyzed by means of a commercial Orsat apparatus. In the results thus obtained (see Table III) some error was caused by replacing with fresh air that withdrawn for analysis; and the temperature of the cool samples rose slowly, while that of the warmer sample fell somewhat during the test. The differences, however, are far too great to leave any doubt as to the facts. With corn at a temperature of 25° (picked near noon on a warm day) there was over 19 per cent carbon dioxid at the end of 4 hours. With corn at a temperature of 15° (picked in the morning) 8 hours were required to reach practically the same point, while with still cooler corn the point was not passed in 10 hours.[1]

TABLE III.—*Oxygen and carbon-dioxid content of air in which green sweetcorn had been stored in a sealed container*

Temperature of corn.	Content of air.	Number of hours after corn was sealed.									
		1	2	3	4	5	6	7	8	9	10
°C.											
10	Oxygen	.....	.....	.....	.....	.....	6	.....	.....	.....	0.2
	Carbon dioxid	.....	.....	.....	.....	.....	10	.....	.....	.....	19.8
15	Oxygen	16.0	12.8	9.5	7.8	6.0	3.6	2.1	0.4	0.0	.....
	Carbon dioxid	3.2	5.2	8.3	10.4	13.0	15.4	17.8	19.6	22.8	.....
25	Oxygen	12.2	8.0	3.2	1.0	.2	.....	.....	.....	.....	.....
	Carbon dioxid	7.0	12.0	16.0	19.8	22.7	.....	.....	.....	.....	.....

TEMPERATURE OF GREEN CORN IN RELATION TO AIR TEMPERATURE

In view of the rapidity with which green corn loses its sugar and the relation of this loss to temperature, it is apparent that if the temperature of the corn itself is near that of the air there must be a difference in the extent of deterioration which would occur during a given interval in different localities and that this difference must correspond to the differences in climatic temperatures. Observations in Maryland and in Maine indicate that the temperature of green corn on the stalk in the shade is usually near that of the air while in the sun it is often well above that of the air (see Table IV).

It will be noted that in the cases cited in the table, which are typical of several others, the corn was 10° or more than 10° C. warmer in the afternoon than in the morning.[2]

[1] It may be of interest to note that the corn which had been kept for some time in an atmosphere deficient in oxygen was of extremely poor quality.

[2] Compare in this connection the condition reported in small fruits (*12*).

TABLE IV.—*Temperature (°C.) of ears of green sweetcorn on the stalk on clear days*

Time.	Glencoe, Md.,[1] Aug. 6, 1918.		Glencoe, Md., Aug. 9, 1918.		Lewiston, Me., Aug. 15, 1918.	
	Air.	Corn.	Air.	Corn.	Air.	Corn.
a. m.						
6	23.6	24.7	19.9	19.6	16.0	15.3
7	24.6	24.7	21.5	22.0	17.0	17.0
8	26.0	25.0	21.3	26.7	18.5	18.5
9	29.5	28.7	28.1	31.2	18.5	22.0
10	32.0	31.6	30.1	35.0	19.0	22.5
11	33.7	32.8	31.5	36.6	20.0	23.5
12	34.5	33.5	32.5	36.7	21.0	24.0
p. m.						
1	.....	.....	33.1	35.3	22.5	25.0
2	34.5	34.5	33.7	35.6	23.0	26.0
3	35.0	34.7	33.8	34.2	22.5	26.0
4	35.3	35.0	33.5	33.5	24.0	26.0
5	34.5	35.6	27.8	30.3	24.0	27.0
6	34.3	35.2	.....	.....	23.5	23.5
7	.....	.....	23.6	24.2	22.0	22.0
8	.....	.....	.....	.....	19.0	19.0

[1] The temperatures in Maryland were taken by Mr. William E. Seifriz.

TEMPERATURE AT CORN-PICKING TIME IN MARYLAND AND IN MAINE

In attempting to study the temperature of different regions in their relation to plant growth the investigator must still depend chiefly on meteorological data taken in cities. Thus Cox (*4, p. 10*), working on so highly specialized a crop as the cranberry, in order to compare the different regions was forced to use temperature readings observed in shelters over hard land, even though his own work had shown the great difference between air temperature over the marshes and air temperature over hard land.

In comparing the temperature of the corn-canning districts of Maryland and Maine, use will be made of the data from the observation stations of the Weather Bureau at Baltimore and Portland. The sweetcorn canning district of Maryland extends from Dorchester County north to Harford County and west to Frederick County. The most important localities lie north and west of Baltimore. In Maine, corn is canned commercially from northern York County to southern Penobscot, the most important localities lying north and west of Portland. It is probable then that the observations at Baltimore and at Portland furnish a fairly reliable index of the difference in temperature between the sweetcorn producing districts of Maryland and Maine. Maryland and Maine were chosen for comparison because they are the most southerly and the most northerly of the important corn-canning districts on the Atlantic seaboard and were among those included in the work of Straughn

and Church. The exact date on which sweetcorn reaches edible condition naturally varies somewhat with different seasons;[1] but corn-canning time in Maryland almost always falls during August, and in Maine during September. In order, then, to give some idea of the temperature conditions under which sweetcorn is handled in the two States, it will be necessary to compare the temperature of Baltimore in August with that of Portland in September.

TABLE V.—*Daily normal temperatures and corresponding indices for Baltimore, Md., August 2 to 31, and Portland, Me., September 1 to 30*

Daily normal temperatures.		Remainder indices.		Exponential indices.		Physiological indices.	
Baltimore.	Portland.	Baltimore.	Portland.	Baltimore.	Portland.	Baltimore.	Portland.
° *F.*	° *F.*	° *F.*	° *F.*				
76	64	37	25	4.0000	2.5198	82.333	30.000
76	63	37	24	4.0000	2.4245	82.333	27.111
76	63	37	24	4.0000	2.4245	82.333	27.111
76	63	37	24	4.0000	2.4245	82.333	27.111
76	63	37	24	4.0000	2.4245	83.333	27.111
76	62	37	23	4.0000	2.3331	82.333	24.333
76	62	37	23	4.0000	2.3331	82.333	24.333
76	62	37	23	4.0000	2.3331	82.333	24.333
76	62	37	23	4.0000	2.3331	83.333	24.333
76	61	37	22	4.0000	2.2451	82.333	22.000
75	61	36	22	3.8480	2.2451	78.111	22.000
75	61	36	22	3.8480	2.2451	78.111	22.000
75	60	36	21	3.8480	2.1603	78.111	19.883
75	60	36	21	3.8480	2.1603	78.111	19.883
75	60	36	21	3.8480	2.1603	78.111	19.883
75	60	36	21	3.8480	2.1603	78.111	19.883
75	59	36	20	3.8480	2.0786	78.111	17.778
74	59	35	20	3.7024	2.0786	73.667	17.778
74	59	35	20	3.7024	2.0786	73.667	17.118
74	58	35	19	3.7024	2.0000	73.667	16.111
74	58	35	19	3.7024	2.0000	73.667	16.111
74	58	35	19	3.7024	2.0000	73.667	16.111
74	57	35	18	3.7024	1.9240	73.667	14.444
73	57	34	18	3.5629	1.9240	69.000	14.444
73	57	34	18	3.5629	1.9240	69.000	14.444
73	56	34	17	3.5629	1.8512	69.000	12.778
73	56	34	17	3.5629	1.8512	69.000	12.778
73	56	34	17	3.5629	1.8512	69.000	12.778
72	55	33	16	3.4283	1.7815	65.333	11.667
72	55	33	16	3.4283	1.7815	65.333	11.667
Av. 74.6	59.5	35.6	20.5	3.7940	2.13504	76.2591	19.5992

Table V gives the daily normal mean temperatures of Baltimore, Md., from August 2 to 31 and of Portland, Me., from September 1 to 30, with three sets of corresponding temperature efficiency indices. The normal mean temperatures are those calculated by Bigelow (*3*) from observed temperatures. That mean temperatures furnish only a very unsatis-

[1] The harvest dates given by Straughn and Church (*14*) are: for Maryland, first week in August, 1905; about the first of August, 1906; about Aug. 15, 1907; and Aug. 23, 1908; for Maine, about Sept. 15, 1905; Sept. 25, 1906; frost before crop matured, 1907; and Sept. 19, 1908.

factory basis for estimating the temperature value for physiological processes of a given climate has long been recognized, and the three sets of indices represent three suggested methods of deriving from mean temperatures some index which would more nearly represent temperature efficiency.

Remainder indices, derived by subtracting a constant quantity (in this case 39) from each daily mean temperature, have been in use for a considerable time. The other methods were suggested recently by Livingston (*7*, *8*), and all three are fully discussed by him in the papers cited.[1]

The exponential system is based on the supposition that plant growth rates follow the chemical principle of van't Hoff and Arrhenius, which states that the velocities of chemical reactions about double with each increase in temperature of 10° C. The physiological indices were calculated from actual temperature values for the growth of corn (maize) seedlings from 10 to 12 mm. high, as worked out by Lehenbauer (*6*). In view of Appleman and Arthur's conclusion (*2*) that the average temperature coefficient of sugar depletion in sweetcorn is about 2, Livingston's "Exponential Indices" based on a coefficient of 2 are of special interest.

The degree of accuracy with which any index derived from mean daily temperatures—½ (maximum + minimum)—expresses the temperature of the day must depend somewhat on the daily temperature range and on the shape of the curve of hourly temperatures. In figure 1 are plotted the curves of normal hourly temperatures for August at Baltimore, as published by Fassig (*5*, *p. 61*), and the mean hourly temperature at Portland for September, 1918.[2] It will be observed that the curves are of the same general shape and that the daily ranges of temperature are similar.

The curves of mean hourly temperatures shown in figure 1 furnish a striking evidence of the difference in the temperatures of the contrasted regions during the corn-packing season. The highest mean temperature at Portland, 62.6° F., is 6° below the lowest mean temperature for Baltimore, 68.6°.

From the purposes of the present paper, however, it is unimportant to determine which method most nearly represents the actual rate of loss, since on whatever basis the comparison is made it is evident that the average day during the corn-packing season in Maryland is much warmer and therefore much more severe in its effect on sweetcorn than the average day of the corresponding season in Maine. Deterioration of corn after picking during a given period would then ordinarily be much

[1] These three kinds of temperature efficiency indices have been compared by one of the writers in connection with studies of the growth of fungi in relation to temperature (*11*).

[2] The normal hourly temperature for Portland has not been computed. The curve for Portland was prepared from data for the month of September, 1918, kindly furnished the writer by Mr. Edward P. Jones, Meteorologist, in charge of the Portland, Me., Station. This, according to advice from Dr. P. C. Day, Chief of Climatological Division, of the U. S. Weather Bureau, gives a fairly representative curve.

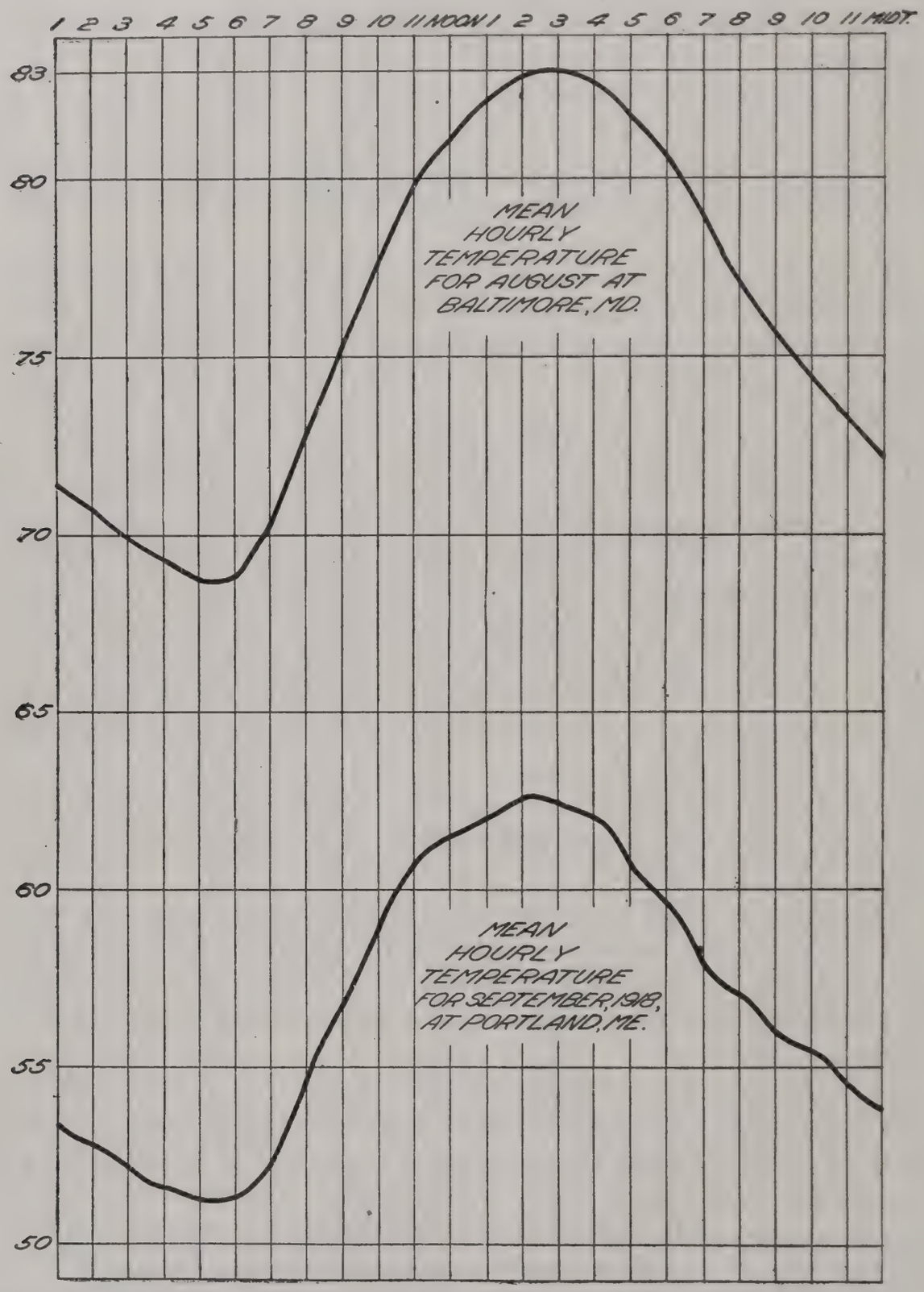
1 2 3 4 5 6 7 8 9 10 11 NOON 1 2 3 4 5 6 7 8 9 10 11 MIDT.
83
80
75
70
65
60
55
50
MEAN HOURLY TEMPERATURE FOR AUGUST AT BALTIMORE, MD.
MEAN HOURLY TEMPERATURE FOR SEPTEMBER, 1918, AT PORTLAND, ME.

FIG. 1.—Mean hourly temperatures for August at Baltimore, Md., and for September, 1918, at Portland, Me.

greater in Maryland than in Maine. The original quality and the methods of handling being equal, corn handled at a mean temperature of 59.5° F., the mean temperature at Portland in September, must inevitably be superior to corn handled at 74.6°, the mean temperature at Baltimore in August.[1]

SUMMARY

The rate at which sugar is lost increases with rise of temperature at least up to 20° C.

The rate of respiration also varies with temperature, being greater at higher temperatures, at least up to 30° C.

Observations in Maryland and in Maine indicate that the temperature of green corn on the stalk while in the shade is usually near that of the air, while in the sun it often is above that of the air.

The corn-picking season in Maryland (August) has a much higher average temperature than the corresponding season (September) in Maine. The difference is sufficient to cause considerably greater deterioration in picked corn during a given period.

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[1] The practical application of the facts here presented in such matters as home canning and handling are too obvious to need comment. As indicated by Table V, corn picked early in the morning is much cooler and can be handled with much less loss of sugar than that picked later in the day.

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VARIATION OF AYRSHIRE COWS IN THE QUANTITY AND FAT CONTENT OF THEIR MILK[1]

By RAYMOND PEARL and JOHN RICE MINER

The present paper has for its purpose a biometrical analysis of the normal individual variation in the milk flow and the fat content of the milk in Ayrshire cattle.

This work has been undertaken because of a strong conviction on the part of the authors that a fairly comprehensive knowledge of the normal variation of a character which is to be made the basis of genetic study is essential if such study is to be critical. This viewpoint is entirely independent of any position which one may hold regarding the genetic significance of different kinds of variation. As a matter of biological fact one never deals actually with one sort of variation absolutely free from the influence or effect of all others. For, even though we may be studying a discontinuous variation of strictly germinal origin and control, there will be, in the actual somatic expression of this variation, a superimposed fluctuating variation of nongerminal origin. The student of genetics ordinarily, and quite rightly, neglects these superimposed fluctuations and confines his attention to the underlying germinal variation.

This is logically a perfectly justifiable procedure, but an essential to its successful operation is that one shall have such an intimate and thorough knowledge of the normal variability of the character in question that he can make his rejections of the unimportant with substantial correctness and hence safety.

These considerations become particularly significant when the character dealt with is one especially subject to environmental influences, in consequence of which the fluctuations assume highly significant proportions in relation to the underlying germinal differences. Such characters are, for example, fecundity, fertility, and, to a marked degree, milk production in cattle. Any milk or fat record represents the result of the action of a complex of factors, of which those classed broadly as environmental certainly play a very important part. To arrive at any sound conclusions regarding the inheritance of these characters it will be essential to form some sort of judgment as to the proportionate parts which genetic and environmental factors play in the production of particular, individual records. It seems perfectly clear that a prerequisite to

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 125.

This work was begun while the authors were actively connected with the Maine Agricultural Experiment Station. It was interrupted by the entry of the United States into the war and has been completed in the Laboratory of Biometry and Vital Statistics of the School of Hygiene and Public Health of Johns Hopkins University.

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anything approaching a sound basis for such a judgment is a thorough analytical study, with the best of biometric tools, of the normal variability of milk and fat production.

MATERIAL FOR INVESTIGATION

The present study is based on the records of Ayshire cattle published in the Reports of the Ayrshire Cattle Milk Records Committee of Scotland, compiled by Speir (*26*) [1] and Howie [2] (*6*). Portions of the very valuable records gathered by this committee have been used by other students of the problems of milk production, notably Wilson (*30*), Pearson (*23*), and most recently Vigor (*28*). Wilson made use of the 1908 records, and Vigor those of 1909 for the Fenwick district only.

The reports under consideration include, so far as it is possible to get the information, the following items:

1. Total milk produced (in gallons).
2. Average percentage of fat, determined from periodic tests.
3. Total milk calculated to a 3 per cent fat basis.
4. Weeks in milk.
5. Age of cow.
6. Date of last calving.
7. Miscellaneous information about the cow, particularly of abnormal circumstances of any sort during the test.

In many cases information is lacking on some one or more of these points, so that, while altogether 8,132 cows were tested in 1908 and 9,202 in 1909, nothing like these numbers are available for analytical study. Another difficulty arises in the fact that there is, of course, much overlapping of calendar years by the lactation periods. Again there is in some districts frequent failure to state the age of the cow.

In the present study all available records from the 1908 and 1909 reports have been used, if they came within the following regulations which we established in order to secure critical material for variation study:

(*a*) The record must be complete in all particulars—that is, cover items 1 to 6 in the list above.

(*b*) The record must be based on 32 or more weeks in milk.

(*c*) There must be nothing of an abnormal or unusual nature about the cow or the lactation, so far as discoverable from the records.

The first of these restrictions requires no comment.

Regarding the second it may be said that the reason for imposing this restriction was that, for present purposes, we desired to use long term averages, rather than to consider lactations of all durations. There

[1] Reference is made by number (italic) to "Literature cited," p. 320–322.

[2] It is a great pleasure to acknowledge, with grateful thanks, the kindness of Mr. John Howie, of Ayr, Scotland, the secretary of the Milk Records Committee, in furnishing a set of the committee's reports for this investigation.

seemed good reason for the belief that one was likely to get better—that is, more nearly physiologically normal—values for the two characters here studied—mean fat percentage and mean weekly yield—if one considered only lactations eight months or more long. Furthermore it is clear that no error of any consequence can be introduced by leaving out of account short lactations, since Vigor (*28*) has shown that there is no significant net correlation between duration of lactation and either percentage of fat or average weekly yield of milk, the two characters studied in this investigation.

The third restriction obviously needs no argument in its justification. Under it were excluded cases of abortion, "off-food" at particular tests, diseases, and accidents of various sorts. Undoubtedly some records were excluded which might fairly have been regarded as normal; but it was thought best, where one was working entirely from records and could not see the cow itself, to err, if at all, on the side of too great rather than too little strictness.

The two characters dealt with in this paper are (*a*) average milk yield per week in gallons, and (*b*) average fat percentage. The values for the former were obtained by dividing the total yields as given in the reports by the weeks in milk. The fat percentage figures were taken directly from the reports. The ages were taken as centering at the mid-point of each year. For example, all cows recorded as 3 years or more in age but less than 4 years were put in the 3-year class in the tables of the present paper. Hence a 3-year-old is to be taken as including anything between 3 and 4 years.

The biometric methods used were the ordinary ones. All of the distributions containing enough individuals to make the results significant were fitted with Pearson's (*18*, *20*) skew frequency curves, following in the computations some simplifications of method.

FREQUENCY DISTRIBUTIONS

The frequency distributions, showing the variation in the two characters studied, are exhibited in Tables I and II, in both absolute and percentage figures.

TABLE I.—*Frequency distributions for variations in average weekly milk yield of Ayrshire cows of different ages*

Yield (in gallons).	2-year-old cows.						3-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
5.00												
5.50												
6.00												
6.50									2	0.24	2	0.14
7.00	1	2.86			1	1.16	3	0.49	3	.36	6	.41
7.50							2	.32	5	.61	7	.48
8.00							2	.32	5	.61	7	.48
8.50	1	2.86	2	3.92	3	3.49	2	.32	3	.36	5	.35
9.00			2	3.92	2	2.33	6	.98	18	2.18	24	1.67
9.50			2	3.92	2	2.33	9	1.46	19	2.30	28	1.94
10.00	1	2.86	2	3.92	3	3.49	14	2.27	21	2.55	35	2.43
10.50			2	3.92	2	2.33	20	3.25	36	4.37	56	3.89
11.00	1	2.86	1	1.96	2	2.33	21	3.41	47	5.70	68	4.72
11.50			7	13.73	7	8.13	34	5.52	36	4.37	70	4.86
12.00	4	11.43	3	5.88	7	8.13	41	6.66	66	8.00	107	7.43
12.50	2	5.71	1	1.96	3	3.49	54	8.77	64	7.76	118	8.19
13.00	6	17.13	4	7.85	10	11.63	54	8.77	70	8.48	124	8.61
13.50	4	11.43	5	9.81	9	10.47	59	9.58	60	7.27	119	8.26
14.00	3	8.57	2	3.92	5	5.81	60	9.74	73	8.85	133	9.23
14.50	1	2.86	1	1.96	2	2.33	36	5.84	51	6.18	87	6.04
15.00	1	2.86	5	9.81	6	6.98	41	6.66	61	7.39	102	7.08
15.50	3	8.57	2	3.92	5	5.81	34	5.52	44	5.33	78	5.41
16.00	2	5.71	3	5.88	5	5.81	35	5.68	41	4.97	76	5.27
16.50	2	5.71	3	5.88	5	5.81	19	3.08	24	2.91	43	2.98
17.00	1	2.86	1	1.96	2	2.33	20	3.25	23	2.79	43	2.98
17.50	1	2.86	2	3.92	3	3.49	21	3.41	7	.85	28	1.94
18.00							7	1.14	13	1.58	20	1.39
18.50			1	1.96	1	1.16	5	.81	17	2.06	22	1.53
19.00	1	2.86			1	1.16	7	1.14	7	.85	14	.97
19.50							2	.32	3	.36	5	.35
20.00							2	.32	4	.48	6	.41
20.50							3	.49			3	.21
21.00							1	.16	1	.12	2	.14
21.50							1	.16	1	.12	2	.14
22.00												
22.50							1	.16			1	.07
23.00												
23.50												
24.00												
24.50												
25.00												
25.50												
Total	35		51		86		616		825		1,441	

TABLE I.—*Frequency distributions for variation in average weekly milk yield of Ayrshire cows of different ages*—Continued

Yield (in gallons).	4-year-old cows.						5-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
5.00												
5.50			1	0.17	1	0.09						
6.00	1	0.19			1	.09						
7.00							1	0.24			1	0.11
7.50			1	.17	1	.09	1	.24			1	.11
8.00	2	.38	1	.17	3	.27			1	0.20	1	.11
8.50	4	.76	4	.68	8	.71			1	.20		
9.00	2	.38	3	.51	5	.45						
9.50	6	1.14	2	.34	8	.71	1	.24	2	.41	3	.33
10.00	6	1.14	8	1.35	14	1.25	2	.47	1	.20	3	.33
10.50	10	1.90	9	1.52	19	1.70	2	.47	2	.41	4	.44
11.00	13	2.47	18	3.04	31	2.77	5	1.19	9	1.85	14	1.54
11.50	16	3.04	26	4.39	42	3.76	3	.71	7	1.44	10	1.10
12.00	30	5.70	18	3.04	48	4.29	8	1.90	10	2.05	18	1.98
12.50	23	4.37	30	5.07	53	4.74	9	2.14	13	2.67	22	2.42
13.00	27	5.13	32	5.40	59	5.28	17	4.04	18	3.69	35	3.85
13.50	25	4.75	45	7.60	70	6.26	18	4.28	17	3.48	35	3.85
14.00	29	5.51	48	8.11	77	6.89	22	5.23	29	5.94	51	5.61
14.50	37	7.04	54	9.12	91	8.14	31	7.36	47	9.63	78	8.59
15.00	41	7.80	39	6.59	80	7.16	33	7.84	29	5.94	62	6.83
15.50	46	8.75	44	7.43	90	8.05	37	8.79	40	8.20	77	8.44
16.00	30	5.70	30	5.07	60	5.37	34	8.08	44	9.02	78	8.59
16.50	31	5.90	42	7.09	73	6.53	35	8.31	31	6.35	66	7.27
17.00	35	6.60	32	5.40	67	5.99	19	4.51	35	7.17	54	5.94
17.50	22	4.18	20	3.38	42	3.76	16	3.80	30	6.15	46	5.06
18.00	30	5.71	17	2.87	47	4.20	30	7.13	21	4.30	51	5.61
18.50	20	3.80	15	2.53	35	3.13	23	5.46	24	4.92	47	5.17
19.00	12	2.28	12	2.03	24	2.14	16	3.80	12	2.46	28	3.08
19.50	13	2.47	7	1.18	20	1.79	18	4.28	18	3.69	36	3.96
20.00	7	1.33	15	2.53	22	1.97	10	2.38	12	2.46	22	2.42
20.50	1	.19	3	.51	4	.36	6	1.42	11	2.26	17	1.87
21.00	1	.19	9	1.52	10	.89	5	1.19	8	1.64	13	1.43
21.50	1	.19			1	.09	5	1.19	2	.41	7	.77
22.00	3	.57	2	.34	5	.45	2	.47	3	.62	5	.55
22.50			1	.17	1	.09	3	.71	4	.82	7	.77
23.00							2	.47	2	.41	4	.44
23.50			3	.51	3	.27	2	.47	1	.20	3	.33
24.00	1	.19			1	.09	2	.47	2	.41	4	.44
24.50									1	.20	1	.11
25.00	1	.19			1	.09	1	.24			1	.11
25.50			1	.17	1	.09	1	.24			1	.11
27.00									1	.20	1	.11
29.00						.11	1	.24			1	.11
Total	526		592		1,118		421		488		909	

TABLE I.—*Frequency distributions for variation in average weekly milk yield of Ayrshire cows of different ages*—Continued

Yield (in gallons).	6-year-old cows.						7-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
8.00	.....	.....	.....	.....	.....	.....	1	0.32	.....	.....	1	0.14
8.50	2	0.61	.....	.....	2	0.25	.....	.....	.....	.....	.....	.....
9.00	1	.31	1	0.21	2	.25	.....	.....	.....	.....	.....	.....
9.50	.....	.....	1	.21	1	.12	.....	.....	.....	.....	.....	.....
10.00	1	.31	3	.63	4	.50	.....	.....	1	0.25	1	.14
10.50	1	.31	1	.21	2	.25	.....	.....	1	.25	1	.14
11.00	2	.61	2	.42	4	.50	1	.32	1	.25	2	.28
11.50	1	.31	1	.21	2	.25	3	.95	3	.76	6	.84
12.00	3	.92	5	1.05	8	1.00	1	.32	1	.25	2	.28
12.50	5	1.53	11	2.31	16	1.99	9	2.22	4	1.01	11	1.54
13.00	6	1.83	10	2.10	16	1.99	4	1.26	7	1.76	11	1.54
13.50	9	2.75	20	4.19	29	3.61	8	2.53	9	2.27	17	2.39
14.00	10	3.05	24	5.03	34	4.23	7	2.22	8	2.02	15	2.12
14.50	16	4.89	30	6.29	46	5.72	10	3.16	21	5.30	31	4.35
15.00	16	4.89	27	5.66	43	5.35	14	4.43	22	5.56	36	5.06
15.50	14	4.28	26	5.45	40	4.98	15	4.75	27	6.82	42	5.92
16.00	20	6.12	46	9.64	66	8.21	21	6.65	23	5.81	44	6.18
16.50	30	9.17	24	5.03	54	6.72	23	7.28	28	7.07	51	7.16
17.00	25	7.65	33	6.92	58	7.21	24	7.59	38	9.60	62	8.71
17.50	16	4.89	35	7.34	51	6.34	8	2.53	31	7.83	39	5.48
18.00	17	5.20	25	5.24	42	5.22	27	8.54	22	5.56	49	6.88
18.50	20	6.12	22	4.61	42	5.22	14	4.43	26	6.57	40	5.62
19.00	26	7.95	21	4.40	47	5.85	20	6.33	25	6.31	45	6.32
19.50	24	7.34	21	4.40	45	5.60	18	5.70	19	4.80	37	5.20
20.00	11	3.36	22	4.61	33	4.10	19	5.38	14	3.54	31	4.35
20.50	13	3.98	19	3.97	32	3.98	15	4.75	18	4.55	33	4.63
21.00	8	2.44	6	1.26	14	1.74	9	2.85	8	2.02	17	2.39
21.50	6	1.83	12	2.52	18	2.24	11	3.48	6	1.51	17	2.39
22.00	8	2.44	8	1.68	16	1.99	13	4.11	9	2.27	22	3.09
22.50	1	.31	5	1.05	6	.75	2	.63	3	.76	5	.70
23.00	6	1.83	1	.21	7	.87	5	1.58	4	1.01	9	1.26
23.50	3	.92	2	.42	5	.62	5	1.58	.....	.....	5	.70
24.00	1	.31	3	.63	4	.50	5	1.58	5	1.26	10	1.40
24.50	2	.61	4	.84	6	.75	3	.95	6	1.52	9	1.26
25.00	1	.31	2	.42	3	.37	.....	.....	1	.25	1	.14
25.50	1	.31	1	.21	2	.25	4	1.26	3	.76	7	.98
26.00	.....	.....	1	.21	1	.12	.....	.....	.....	.....	.....	.....
26.50	1	.31	.....	.....	1	.12	.....	.....	.....	.....	.....	.....
27.00	.....	.....	1	.21	1	.12	1	.32	1	.25	2	.28
29.00	.....	.....	1	.21	1	.12	.....	.....	.....	.....	.....	.....
30.50	.....	.....	.....	.....	.....	.....	.....	.....	1	.25	1	.14
Total	327	.....	477	.....	804	.....	316	.....	396	.....	712	.....

TABLE I.—*Frequency distributions for variation in average weekly milk yield of Ayrshire cows of different ages*—Continued

Yield (in gallons).	8-year-old cows.						9-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
8.50	1	0.37	.....	.....	1	0.16	.....	.....	.....	.....	.....	.....
9.50	2	.74	.....	.....	2	.31	.....	.....	.....	.....	.....	.....
11.00	.....	.....	1	0.27	1	.16	1	0.47	1	0.40	2	0.44
11.50	1	.37	2	.55	3	.47	2	.95	1	.40	3	.65
12.00	1	.37	8	2.18	9	1.41	.....	.....	2	.81	2	.44
12.50	4	1.48	1	.27	5	.78	1	.47	2	.81	3	.65
13.00	6	2.22	1	.27	7	1.10	1	.47	1	.40	2	.44
13.50	4	1.48	4	1.09	8	1.26	4	1.89	7	2.84	11	2.40
14.00	12	4.45	15	4.09	27	4.24	6	2.83	7	2.84	13	2.83
14.50	5	1.85	10	2.73	15	2.35	5	2.36	6	2.43	11	2.40
15.00	12	4.45	14	3.82	26	4.08	7	3.30	14	5.67	21	4.58
15.50	9	3.33	25	6.81	34	5.34	7	3.30	10	4.05	17	3.70
16.00	22	8.15	18	4.90	40	6.28	10	4.72	14	5.67	24	5.23
16.50	15	5.56	20	5.45	35	5.49	14	6.60	13	5.26	27	5.88
17.00	23	8.52	29	7.90	52	8.16	12	5.66	23	9.31	35	7.63
17.50	13	4.81	28	7.63	41	6.44	25	11.79	16	6.48	41	8.93
18.00	22	8.15	25	6.81	47	7.38	12	5.66	16	6.48	28	6.10
18.50	20	7.41	26	7.08	46	7.22	16	7.55	15	6.07	31	6.75
19.00	14	5.19	20	5.45	34	5.34	14	6.60	12	4.86	26	5.66
19.50	22	8.15	15	4.09	37	5.81	6	2.83	16	6.48	22	4.79
20.00	19	7.04	17	4.63	36	5.65	13	6.13	7	2.84	20	4.36
20.50	6	2.22	22	5.99	28	4.40	14	6.60	10	4.05	24	5.23
21.00	6	2.22	17	4.63	23	3.61	7	3.30	16	6.48	23	5.01
21.50	6	2.22	8	2.18	14	2.20	7	3.30	11	4.45	18	3.92
22.00	6	2.22	6	1.64	12	1.88	9	4.25	3	1.21	12	2.61
22.50	3	1.11	10	2.73	13	2.04	4	1.89	5	2.03	9	1.96
23.00	4	1.48	6	1.64	10	1.57	5	2.36	7	2.84	12	2.61
23.50	3	1.11	7	1.91	10	1.57	2	.95	6	2.43	8	1.74
24.00	2	.74	2	.55	4	.63	1	.47	1	.40	2	.44
24.50	2	.74	5	1.36	7	1.10	2	.95	1	.40	3	.65
25.00	.....	.....	1	.27	1	.16	1	.47	3	1.21	4	87
25.50	.....	.....	.....	.....	.....	.....	1	.47	.....	.....	1	.22
26.00	1	.37	1	.27	2	.31	.....	.....	1	.40	1	.22
26.50	3	1.11	1	.27	4	.63	1	.47	.....	.....	1	.22
27.00	.....	.....	.....	.....	.....	.....	1	.47	.....	.....	1	.22
27.50	1	.37	1	.27	2	.31	.....	.....	.....	.....	.....	.....
28.50	.....	.....	.....	.....	.....	.....	1	.47	.....	.....	1	.22
29.00	.....	.....	1	.27	1	.16	.....	.....	.....	.....	.....	.....
Total.....	270	.....	367	.....	637	.....	212	.....	247	.....	459	.....

TABLE I.—*Frequency distributions for variation in average weekly milk yield of Ayrshire cowsof different ages*—Continued

Yield (in gallons).	10-year-old cows.						11-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
7.00									1	0.93	1	0.50
7.50												
8.00							1	1.06			1	.50
9.00							1	1.06	1	.93	2	.99
10.00	1	0.79			1	0.31						
11.00			1	0.52	1	.31	1	1.06	1	.93	2	.99
11.50	1	.79	2	1.03	3	.94	1	1.06			1	.50
12.00	1	.79			1	.31	2	2.13			2	.99
12.50	1	.79			1	.31	1	1.06	1	.93	2	.99
13.00	1	.79	2	1.03	3	.94			1	.93	1	.50
13.50	3	2.38	6	3.09	9	2.81	3	3.19	4	3.70	7	3.46
14.00	2	1.59	4	2.06	6	1.88	3	3.19	2	1.85	5	2.47
14.50	5	3.97	3	1.54	8	2.50	2	2.13	2	1.85	4	1.98
15.00	4	3.17	8	4.12	12	3.75	2	2.13	10	9.26	12	5.94
15.50	1	.79	6	3.09	7	2.19	4	4.26	6	5.55	10	4.95
16.00	5	3.97	11	5.67	16	5.00	4	4.26	4	3.70	8	3.96
16.50	12	9.53	16	8.25	28	8.75	7	7.45	9	8.33	16	7.92
17.00	10	7.94	9	4.64	19	5.94	4	4.26	8	7.41	12	5.94
17.50	10	7.94	12	6.19	22	6.88	6	6.39	9	8.33	15	7.43
18.00	7	5.56	19	9.79	26	8.12	3	3.19	4	3.70	7	3.46
18.50	5	3.97	7	3.61	12	3.75	8	8.51	6	5.55	14	6.93
19.00	5	3.97	12	6.19	17	5.31	8	8.51	8	7.41	16	7.92
19.50	11	8.73	9	4.64	20	6.25	5	5.32	7	6.48	12	5.94
20.00	4	3.17	10	5.15	14	4.38	6	6.39	4	3.70	10	4.95
20.50	6	4.77	12	6.19	18	5.62	6	6.39	5	4.63	11	5.44
21.00	7	5.56	9	4.64	16	5.00	1	1.06	3	2.78	4	1.98
21.50	7	5.56	9	4.64	16	5.00	1	1.06	3	2.78	4	1.98
22.00	5	3.97	6	3.09	11	3.44	3	3.19	2	1.85	5	2.47
22.50	4	3.17	8	4.12	12	3.75	2	2.13			2	.99
23.00	3	2.38	4	2.06	7	2.19	1	1.06	2	1.85	3	1.48
23.50	1	.79	2	1.03	3	.94	2	2.13	2	1.85	4	1.98
24.00	1	.79	1	.52	2	.62	1	1.06	1	.93	2	.99
24.50			1	.52	1	.31	2	2.13	1	.93	3	1.48
25.00							1	1.06			1	.50
25.50									1	.93	1	.50
26.00	1	.79	2	1.03	3	.94						
26.50	2	1.59	2	1.03	4	1.25						
27.00							1	1.06			1	.50
28.00							1	1.06			1	.50
29.00			1	.52	1	.31						
Total	126		194		320		94		108		202	

TABLE I.—*Frequency distributions for variation in average weekly milk yield of Ayrshire cows of different ages*—Continued

Yield (in gallons).	12-year-old cows.						13-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
10.50			1	1.52	1	0.86						
12.00			1	1.52	1	.86	1	4.76			1	1.61
12.50									1	2.44	1	1.61
13.00	1	2.00	1	1.52	2	1.72	1	4.76			1	1.61
13.50			1	1.52	1	.86			2	4.88	2	3.23
14.00			2	3.03	2	1.72			2	4.88	2	3.23
14.50	2	4.00	2	3.03	4	3.45						
15.00	4	8.00	2	3.03	6	5.17						
15.50	3	6.00	3	4.54	6	5.17			3	7.32	3	4.84
16.00	4	8.00	6	9.09	10	8.62	2	9.52	3	7.32	5	8.06
16.50	2	4.00	8	12.12	10	8.62			1	2.44	1	1.61
17.00	4	8.00	4	6.06	8	6.90	1	4.76	3	7.32	4	6.45
17.50	5	10.00	6	9.09	11	9.49			2	4.88	2	3.23
18.00	1	2.00	3	4.54	4	3.45	3	14.29	4	9.75	7	11.30
18.50	1	2.00	4	6.06	5	4.31	2	9.52	2	4.88	4	6.45
19.00	3	6.00	1	1.52	4	3.45	3	14.29	3	7.32	6	9.68
19.50	6	12.00	1	1.52	7	6.04	2	9.52	1	2.44	3	4.84
20.00	2	4.00	2	3.03	4	3.45			3	7.32	3	4.84
20.50	2	4.00	3	4.54	5	4.31			2	4.88	2	3.23
21.00	2	4.00	5	7.56	7	6.04	2	9.52	3	7.32	5	8.06
21.50	1	2.00	2	3.03	3	2.59	2	9.52	1	2.44	3	4.84
22.00			3	4.54	3	2.59			1	2.44	1	1.61
22.50									2	4.88	2	3.23
23.00	3	6.00	2	3.03	5	4.31	1	4.76			1	1.61
23.50			1	1.52	1	.86			1	2.44	1	1.61
24.00	1	2.00			1	.86						
24.50	1	2.00	1	1.52	2	1.72						
25.50	1	2.00	1	1.52	2	1.72	1	4.76			1	1.61
26.50									1	2.44	1	1.61
28.00	1	2.00			1	.86						
Total	50		66		116		21		41		62	

Yield (in gallons).	14-year-old cows.						15-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
9.00									1	5.26	1	4.76
9.50	1	4.55			1	2.50						
11.00	1	4.55			1	2.50						
12.50									1	5.26	1	4.76
13.00	1	4.55	2	11.11	3	7.50						
13.50									1	5.26	1	4.76
14.00			2	11.11	2	5.00						
14.50	1	4.55	1	5.56	2	5.00						
15.00			1	5.56	1	2.50						
16.00	1	4.55	1	5.56	2	5.00			1	5.26	1	4.76
16.50	1	4.55	2	11.11	3	7.50			2	10.53	2	9.53
17.00	3	13.63			3	7.50			1	5.26	1	4.76
17.50	1	4.55	2	11.11	3	7.50	1	50	2	10.53	3	14.29
18.00	2	9.09	1	5.56	3	7.50						
18.50	3	13.63			3	7.50			3	15.79	3	14.29
19.00	1	4.55			1	2.50			1	5.26	1	4.76
19.50	1	4.55	1	5.56	2	5.00			1	5.26	1	4.76
20.00	1	4.55	1	5.56	2	5.00			2	10.53	2	9.53
20.50	2	9.09	1	5.56	3	7.50			1	5.26	1	4.76
21.00									1	5.26	1	4.76
21.50									1	5.26	1	4.76
23.00			1	5.56	1	2.50						
23.50			1	5.56	1	2.50						
24.00	1	4.55			1	2.50						
25.00	1	4.55			1	2.50	1	50			1	4.76
27.00			1	5.56	1	2.50						
Total	22		18		40		2		19		21	

TABLE I.—*Frequency distributions for variation in average weekly milk yield of Ayrshire cows of different ages*—Continued

Yield (in gallons).	16-year-old cows.					
	1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
14.50			1	16.7	1	12.5
15.50			1	16.7	1	12.5
16.00	1	50.0			1	12.5
17.00			1	16.7	1	12.5
17.50			1	16.7	1	12.5
18.00			1	16.7	1	12.5
20.50	1	50.0			1	12.5
22.00			1	16.7	1	12.5
Total	2		6		8	

TABLE II.—*Frequency distributions for variation in percentage of fat in the milk of Ayrshire cows*

Fat percentage.	2-year-old cows.						3-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
2.85							2	0.33			2	0.14
2.95							2	.33	1	0.12	3	.21
3.05							5	.81	5	.61	10	.69
3.15			1	1.96	1	1.16	12	1.95	16	1.94	28	1.94
3.25			1	1.96	1	1.16	17	2.76	17	2.06	34	2.36
3.35	4	11.43	6	11.77	10	11.63	19	3.08	38	4.61	57	3.96
3.45	1	2.86	4	7.84	5	5.82	45	7.31	53	6.42	98	6.80
3.55	1	2.86	4	7.84	5	5.82	52	8.44	72	8.73	124	8.61
3.65	2	5.71	6	11.77	8	9.30	57	9.25	86	10.42	143	9.92
3.75	8	22.86	4	7.84	12	13.95	65	10.55	92	11.15	157	10.90
3.85	3	8.57	6	11.77	9	10.47	68	11.04	90	10.90	158	10.96
3.95	3	8.57	7	13.73	10	11.63	71	11.53	107	12.97	178	12.35
4.05	6	17.14	2	3.92	8	9.30	43	6.98	64	7.76	107	7.43
4.15	3	8.57	5	9.80	8	9.30	44	7.14	65	7.88	109	7.56
4.25	1	2.86	3	5.88	4	4.65	38	6.17	42	5.09	80	5.55
4.35	2	5.71	1	1.96	3	3.49	19	3.08	27	3.27	46	3.19
4.45			1	1.96	1	1.16	17	2.76	20	2.43	37	2.57
4.55	1	2.86			1	1.16	7	1.14	10	1.21	17	1.18
4.65							16	2.60	12	1.46	28	1.94
4.75							8	1.30	2	.24	10	.69
4.85							4	.65	4	.49	8	.56
4.95							1	.16	1	.12	2	.14
5.15							1	.16	1	.12	2	.14
5.45							1	.16			1	.07
6.35							1	.16			1	.07
6.65							1	.16			1	.07
Total	35		51		86		616		825		1,441	

TABLE II.—*Frequency distributions for variation in percentage of fat in the milk of Ayrshire cows*—Continued

Fat percentage.	4-year-old cows.						5-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
2.75	.....	.....	1	0.17	1	0.09	2	0.47	2	0.41	4	0.44
2.85	6	1.14	2	.34	8	.71	1	.24	4	.82	5	.55
2.95	2	.38	4	.68	6	.53	2	.47	7	1.43	9	.99
3.05	6	1.14	7	1.18	13	1.16	13	3.09	11	2.25	24	2.64
3.15	15	2.85	8	1.35	23	2.06	21	4.99	18	3.69	39	4.29
3.25	24	4.56	32	5.41	56	5.01	18	4.28	24	4.92	42	4.62
3.35	46	8.75	34	5.74	80	7.16	31	7.36	47	9.63	78	8.58
3.45	48	9.13	53	8.95	101	9.03	38	9.03	44	9.02	82	9.02
3.55	59	11.22	65	10.98	124	11.09	52	12.35	68	13.93	120	13.21
3.65	56	10.65	76	12.84	132	11.81	39	9.26	62	12.70	101	11.11
3.75	59	11.22	79	13.34	138	12.34	50	11.88	52	10.66	102	11.22
3.85	60	11.41	62	10.47	122	10.91	41	9.74	46	9.43	87	9.57
3.95	39	7.41	56	9.46	95	8.50	33	7.84	41	8.40	74	8.14
4.05	35	6.65	28	4.73	63	5.64	23	5.46	24	4.92	47	5.17
4.15	22	4.18	32	5.41	54	4.83	23	5.46	14	2.87	37	4.07
4.25	20	3.80	24	4.05	44	3.94	16	3.80	7	1.43	23	2.53
4.35	6	1.14	11	1.86	17	1.52	4	.95	7	1.43	11	1.21
4.45	5	.95	9	1.52	14	1.25	4	.95	2	.41	6	.66
4.55	10	1.90	6	1.01	16	1.43	5	1.19	3	.62	8	.88
4.65	4	.76	1	.17	5	.45	5	1.19	2	.41	7	.77
4.75	2	.38	1	.17	3	.27	.....	.....	3	.62	3	.33
4.95	1	.19	.....	.....	1	.09	.....	.....	.....	.....	.....	.....
5.15	1	.19	.....	.....	1	.09	.....	.....	.....	.....	.....	.....
6.25	.....	.....	1	.17	1	.09	.....	.....	.....	.....	.....	.....
Total	526	.....	592	.....	1,118	.....	421	.....	488	.....	909	.....

Fat percentage.	6-year-old cows.						7-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
2.75	1	0.31	3	0.63	4	0.50	.....	.....	.....	.....	.....	.....
2.85	2	.61	2	.42	4	.50	.....	.....	.....	.....	.....	.....
2.95	2	.61	4	.84	6	.75	1	0.32	4	1.01	5	0.70
3.05	3	.92	10	2.09	13	1.62	8	2.53	6	1.52	14	1.97
3.15	16	4.89	19	3.98	35	4.35	13	4.11	23	5.80	36	5.05
3.25	17	5.20	35	7.34	52	6.47	20	6.33	20	5.05	40	5.62
3.35	25	7.65	45	9.43	70	8.70	34	10.76	34	8.59	68	9.55
3.45	37	11.31	62	13.00	99	12.31	34	10.76	40	10.10	74	10.39
3.55	44	13.46	57	11.95	101	12.56	39	12.34	50	12.63	89	12.50
3.65	42	12.84	56	11.74	98	12.18	36	11.39	49	12.37	85	11.94
3.75	31	9.48	50	10.48	81	10.07	38	12.03	46	11.61	84	11.80
3.85	31	9.48	45	9.43	76	9.45	30	9.49	45	11.36	75	10.53
3.95	38	11.62	35	7.34	73	9.08	21	6.65	27	6.82	48	6.74
4.05	14	4.28	18	3.77	32	3.98	18	5.70	24	6.06	42	5.90
4.15	11	3.37	14	2.94	25	3.11	9	2.85	11	2.78	20	2.81
4.25	6	1.83	6	1.26	12	1.49	6	1.90	10	2.52	16	2.25
4.35	2	.61	7	1.47	9	1.12	3	.94	3	.76	6	.84
4.45	.....	.....	8	1.68	8	1.00	5	1.58	2	.51	7	.99
4.55	3	.92	.....	.....	3	.38	1	.32	.....	.....	1	.14
4.65	2	.61	1	.21	3	.38	.....	.....	.....	.....	.....	.....
Total	327	.....	477	.....	804	.....	316	.....	396	.....	712	.....

TABLE II.—*Frequency distributions for variation in percentage of fat in the milk of Ayrshire cows*—Continued

Fat percentage.	8-year-old cows.						9-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
2.55	.....	.....	.....	.....	.....	.....	1	0.47	.....	.....	1	0.22
2.75	1	0.37	1	0.27	2	0.31	.....	.....	.....	.....	.....	.....
2.85	2	.74	.....	.....	2	.31	1	.47	1	0.40	2	.44
2.95	3	1.11	2	.55	5	.78	5	2.36	.....	.....	5	1.09
3.05	9	3.33	8	2.18	17	2.67	7	3.30	6	2.43	13	2.83
3.15	8	2.96	18	4.90	26	4.08	8	3.77	14	5.67	22	4.79
3.25	17	6.30	32	8.72	49	7.69	18	8.49	13	5.26	31	6.75
3.35	25	9.26	42	11.44	67	10.52	26	12.26	27	10.93	53	11.55
3.45	35	12.97	39	10.63	74	11.61	30	14.15	38	15.39	68	14.81
3.55	36	13.34	47	12.80	83	13.09	23	10.85	31	12.55	54	11.77
3.65	32	11.85	39	10.63	71	11.14	22	10.38	37	14.97	59	12.86
3.75	32	11.85	36	9.81	68	10.67	20	9.44	21	8.50	41	8.93
3.85	25	9.26	36	9.81	61	9.57	11	5.19	16	6.48	27	5.88
3.95	15	5.56	19	5.18	34	5.34	19	8.96	16	6.48	35	7.63
4.05	16	5.92	20	5.45	36	5.65	12	5.66	14	5.67	26	5.66
4.15	5	1.85	11	3.00	16	2.51	6	2.83	7	2.84	13	2.83
4.25	6	2.22	9	2.45	15	2.35	3	1.42	2	.81	5	1.09
4.35	1	.37	3	.82	4	.63	.....	.....	3	1.22	3	.65
4.45	2	.74	1	.27	3	.47	.....	.....	.....	.....	.....	.....
4.55	.....	.....	1	.27	1	.15	.....	.....	.....	.....	.....	.....
4.65	.....	.....	2	.55	2	.31	.....	.....	1	.40	1	.22
4.75	.....	.....	1	.27	1	.15	.....	.....	.....	.....	.....	.....
Total	270	.....	367	.....	637	.....	212	.....	247	.....	459	.....

Fat percentage.	10-year-old cows.						11-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
2.85	1	0.79	1	0.52	2	0.62	.....	.....	.....	.....	.....	.....
2.95	4	3.17	2	1.03	6	1.87	1	1.06	4	3.70	5	2.47
3.05	2	1.59	3	1.54	5	1.58	2	2.13	2	1.85	4	1.98
3.15	9	7.14	15	7.73	24	7.50	3	3.19	9	8.33	12	5.94
3.25	12	9.53	17	8.77	29	9.06	8	8.51	8	7.41	16	7.92
3.35	12	9.53	20	10.31	32	10.00	13	13.83	12	11.11	25	12.38
3.45	20	15.87	29	14.95	49	15.31	17	18.08	13	12.04	30	14.85
3.55	10	7.94	27	13.92	37	11.56	6	6.39	11	10.18	17	8.41
3.65	16	12.70	23	11.85	39	12.19	12	12.76	13	12.04	25	12.38
3.75	17	13.49	23	11.85	40	12.50	7	7.45	12	11.11	19	9.41
3.85	6	4.77	13	6.70	19	5.94	7	7.45	9	8.33	16	7.92
3.95	8	6.35	9	4.64	17	5.31	6	6.39	7	6.48	13	6.44
4.05	5	3.96	4	2.06	9	2.81	4	4.25	3	2.78	7	3.46
4.15	1	.79	6	3.09	7	2.19	5	5.32	3	2.78	8	3.96
4.25	2	1.59	1	.52	3	.94	2	2.13	1	.93	3	1.48
4.35	.....	.....	.....	.....	.....	.....	.....	.....	1	.93	1	.50
4.45	.....	.....	1	.52	1	.31	.....	.....	.....	.....	.....	.....
4.55	.....	.....	.....	.....	.....	.....	1	1.06	.....	.....	1	.50
4.65	1	.79	.....	.....	1	.31	.....	.....	.....	.....	.....	.....
Total	126	.....	194	.....	320	.....	94	.....	108	.....	202	.....

TABLE II.—*Frequency distributions for variation in percentage of fat in the milk of Ayrshire cows*—Continued

Fat percentage.	12-year-old cows.						13-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
2.85	1	2.00	3	4.54	4	3.45	.....	.....	.....	.....	.....	.....
2.95	.....	.....	1	1.52	1	.86	.....	.....	1	2.44	1	1.61
3.05	2	4.00	1	1.52	3	2.59	1	4.76	3	7.32	4	6.45
3.15	4	8.00	3	4.54	7	6.04	1	4.76	.....	.....	1	1.61
3.25	4	8.00	6	9.09	10	8.62	.....	.....	6	14.63	6	9.68
3.35	4	8.00	9	13.64	13	11.20	.....	.....	2	4.88	2	3.23
3.45	5	10.00	10	15.15	15	12.93	2	9.52	8	19.51	10	16.13
3.55	7	14.00	7	10.61	14	12.07	7	33.34	9	21.95	16	25.80
3.65	7	14.00	9	13.64	16	13.79	3	14.29	4	9.75	7	11.30
3.75	7	14.00	3	4.54	10	8.62	2	9.52	2	4.88	4	6.45
3.85	4	8.00	3	4.54	7	6.04	2	9.52	3	7.32	5	8.06
3.95	1	2.00	4	6.06	5	4.31	.....	.....	.....	.....	.....	.....
4.05	1	2.00	3	4.54	4	3.45	1	4.76	1	2.44	2	3.23
4.15	2	4.00	2	3.03	4	3.45	1	4.76	2	4.88	3	4.84
4.35	.....	.....	1	1.52	1	.86	1	4.76	.....	.....	1	1.61
4.45	1	2.00	1	1.52	2	1.72	.....	.....	.....	.....	.....	.....
Total	50	.....	66	.....	116	.....	21	.....	41	.....	62	.....

Fat percentage.	14-year-old cows.						15-year-old cows.					
	1908.		1909.		Combined years.		1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
3.05	.....	.....	2	11.11	2	5.00	.....	.....	.....	.....	.....	.....
3.15	2	9.09	2	11.11	4	10.00	.....	.....	3	15.79	3	14.29
3.25	2	9.09	1	5.56	3	7.50	.....	.....	1	5.26	1	4.76
3.35	4	18.18	2	11.11	6	15.00	1	50.00	4	21.06	5	23.81
3.45	3	13.64	1	5.56	4	10.00	.....	.....	4	21.06	4	19.05
3.55	3	13.64	3	16.66	6	15.00	1	50.00	5	26.31	6	28.57
3.65	2	9.09	1	5.56	3	7.50	.....	.....	.....	.....	.....	.....
3.75	2	9.09	3	16.66	5	12.50	.....	.....	1	5.26	1	4.76
3.95	3	13.64	2	11.11	5	12.50	.....	.....	.....	.....	.....	.....
4.15	1	4.54	.....	.....	1	2.50	.....	.....	1	5.26	1	4.76
4.45	.....	.....	1	5.56	1	2.50	.....	.....	.....	.....	.....	.....
Total	22	.....	18	.....	40	.....	2	.....	19	.....	21	.....

Fat percentage.	16-year-old cows.					
	1908.		1909.		Combined years.	
	Frequency.	Percentage.	Frequency.	Percentage.	Frequency.	Percentage.
3.35	.....	.....	1	16.67	1	12.50
3.45	.....	.....	1	16.67	1	12.50
3.55	1	50.00	1	16.67	2	25.00
3.65	.....	.....	2	33.33	2	25.00
3.75	.....	.....	1	16.67	1	12.50
3.95	1	50.00	.....	.....	1	12.50
Total	2	.....	6	.....	8	.....

VARIATION CONSTANTS

Before undertaking any discussion of the distributions given in Tables I and II it is desirable to have at hand the simple physical constants, means, standard deviations, and coefficients of variation deduced from them. These constants are accordingly given in Tables III and IV. In the calculation of the standard deviations Sheppard's correction of the second moment was used in all cases.

TABLE III.—*Constants for variation in weekly milk yield*

Age of cow.	Year.	Mean weekly yield (in gallons).	Standard deviation (in gallons).	Coefficient of variation.
2 years	1908	13.907±0.274	2.400±0.193	17.260±1.432
	1909	13.407± .243	2.569± .172	19.165±1.326
	Combined	13.610± .183	2.514± .129	18.471± .980
3 years	1908	14.029± .065	2.392± .046	17.053± .337
	1909	13.701± .058	2.480± .041	18.103± .310
	Combined	13.841± .044	2.449± .031	17.690± .229
4 years	1908	15.289± .080	2.734± .057	17.885± .384
	1909	15.177± .076	2.750± .054	18.118± .367
	Combined	15.230± .055	2.743± .039	18.012± .266
5 years	1908	16.558± .092	2.798± .065	16.899± .572
	1909	16.382± .083	2.715± .059	16.575± .368
	Combined	16.463± .062	2.755± .044	16.736± .272
6 years	1908	17.698± .109	2.933± .077	16.575± .449
	1909	17.314± .092	2.972± .065	17.163± .385
	Combined	17.470± .071	2.962± .050	16.955± .294
7 years	1908	18.278± .117	3.079± .083	16.846± .464
	1909	17.866± .098	2.877± .069	16.103± .397
	Combined	18.049± .075	2.970± .053	16.456± .322
8 years	1908	18.109± .122	2.984± .086	16.477± .491
	1909	18.371± .104	2.952± .074	16.071± .410
	Combined	18.260± .079	2.969± .056	16.258± .315
9 years	1908	18.698± .135	2.911± .095	15.566± .523
	1909	18.434± .125	2.901± .088	15.739± .489
	Combined	18.556± .092	2.909± .065	15.675± .358
10 years	1908	18.683± .183	3.049± .130	16.318± .712
	1909	18.773± .147	3.030± .104	16.138± .566
	Combined	18.738± .115	3.037± .081	16.210± .444
11 years	1908	18.367± .250	3.593± .177	19.560± .999
	1909	17.889± .199	3.059± .140	17.101± .808
	Combined	18.111± .158	3.330± .112	18.384± .638
12 years	1908	18.790± .300	3.143± .212	16.726±1.160
	1909	18.205± .251	3.025± .178	16.617±1.002
	Combined	18.457± .193	3.087± .137	16.723± .761
13 years	1908	19.036± .441	2.995± .312	15.773±1.682
	1909	18.616± .313	2.968± .221	15.943±1.218
	Combined	18.750± .256	2.986± .181	15.924± .989
14 years	1908	17.932± .505	3.509± .357	19.566±2.065
	1909	17.972± .603	3.795± .427	21.116±2.477
	Combined	17.950± .388	3.640± .274	20.280±1.591
15 years	1908	21.500±1.787	3.747±1.264	17.429±6.053
	1909	17.776± .471	3.046± .333	17.138±1.929
	Combined	18.131± .487	3.306± .344	18.234±1.958
16 years	1908	18.500±1.071	2.245± .757	12.137±4.153
	1909	17.500± .658	2.389± .465	13.653±2.707
	Combined	17.875± .564	2.364± .399	13.225±2.269
Weighted means (total)		16.489	2.806	17.081

TABLE IV.—*Constants for variation in percentage of fat in the milk of Ayrshire cows.*

Age of cow.	Year.	Mean fat percentage.	Standard deviation (in percentage).	Coefficient of variation.
2 years	1908	3.891±0.033	0.292±0.024	7.496±0.611
	1909	3.825± .030	.313± .021	8.180± .552
	Combined	3.852± .023	.311± .016	8.080± .415
3 years	1908	[a] 3.924± .011	[a] .412± .008	[a] 10.500± .202
		[b] 3.915± .011	[b] .384± .007	[b] 9.816± .191
	1909	3.895± .008	.344± .006	8.820± .148
	Combined	3.903± .006	.361± .005	9.260± .118
4 years	1908	3.770± .011	.359± .008	9.513± .200
	1909	[c] 3.785± .010	[c] .342± .007	[c] 9.034± .179
		[d] 3.780± .009	[d] .326± .006	[d] 8.626± .171
	Combined	3.775± .007	.342± .005	9.055± .131
5 years	1908	3.741± .012	.358± .008	9.579± .225
	1909	3.694± .010	.335± .007	9.074± .198
	Combined	3.716± .008	.346± .006	9.320± .149
6 years	1908	3.705± .012	.314± .008	8.481± .225
	1909	3.672± .010	.322± .007	8.772± .193
	Combined	3.685± .008	.319± .005	8.664± .147
7 years	1908	3.694± .012	.312± .008	8.448± .228
	1909	3.689± .010	.305± .008	8.259± .200
	Combined	3.691± .008	.308± .006	8.334± .150
8 years	1908	3.658± .013	.306± .009	8.358± .244
	1909	3.668± .011	.323± .008	8.801± .221
	Combined	3.664± .008	.316± .006	8.617± .164
9 years	1908	3.618± .014	.313± .010	8.640± .285
	1909	3.652± .013	.294± .009	8.047± .245
	Combined	3.636± .010	.303± .007	8.336± .187
10 years	1908	3.599± .018	.308± .013	8.569± .367
	1909	3.601± .013	.278± .009	7.732± .266
	Combined	3.600± .011	.291± .008	8.072± .217
11 years	1908	3.653± .022	.312± .015	8.552± .424
	1909	3.608± .020	.306± .014	8.480± .392
	Combined	3.629± .015	.310± .010	8.536± .289
12 years	1908	3.610± .027	.286± .019	7.936± .538
	1909	3.591± .028	.338± .020	9.412± .557
	Combined	3.599± .020	.326± .014	9.066± .405
13 years	1908	3.700± .042	.288± .030	7.785± .815
	1909	3.559± .029	.273± .020	7.684± .576
	Combined	3.606± .024	.286± .017	7.942± .487
14 years	1908	3.595± .039	.271± .028	7.527± .770
	1909	3.589± .056	.353± .040	9.835±1.116
	Combined	3.592± .033	.310± .023	8.641± .656
15 years	1908	3.500± .046	.096± .032	2.736± .923
	1909	3.500± .035	.225± .025	6.436± .707
	Combined	3.500± .032	.216± .022	6.180± .646
16 years	1908	3.800± .094	.198± .067	5.208±1.761
	1909	3.617± .036	.131± .026	3.628± .707
	Combined	3.662± .040	.170± .029	4.650± .786
Weighted means (total)		3.738	.330	8.827

[a] Including the two very high testing cows.
[b] Without the two very high testing cows.
[c] Including the one very high testing cow.
[d] Without the one very high testing cow.

From these tables a number of points are to be noted.

1. It is evident that the mean weekly yield and the fat percentage change with the age of the cow. The nature of these changes will not, however, be discussed here but will be analyzed in detail in a later section of the paper.

2. From comparison of the results here given with those of Vigor (*28*) it is seen that while, the general, the agreement is fairly close, there are some rather striking differences. Taking weighted means from Tables III and IV, we see that the mean weekly yield is slightly lower, and the mean fat percentage slightly higher, in the whole group than in the Fenwick district data alone. The differences in the means, however, are small and probably of no significance. When we turn to variation as measured by standard deviations, there is a striking difference in weekly yield. The weighted mean standard deviation for the whole group is 2.806 gallons, while Vigor finds for the Fenwick district alone 4.0704 gallons. This is a large and statistically significant difference. In fat percentage the standard deviations are practically alike for the two sets of data, our weighted mean value being 0.330 and Vigor's 0.3229

3. The explanation for the difference in variability in weekly yield between our figures and Vigor's is not far to seek. It lies mainly in the fact that Vigor has dealt with cows of all ages lumped together, while in the present paper each year of age is dealt with separately. Naturally when dealing with a character which changes with age so extensively as does milk yield, as has recently been discussed by Pearl (*12*), the variation exhibited will be markedly increased if animals of all ages are lumped together. In order to determine how much of the difference in variation was due to this cause and how much to other factors Table V has been prepared. This table gives the distribution for weekly yield obtained by adding together all of the "combined" distributions for the several years, as set forth in Table I.

TABLE V.—*Distribution for weekly yield combined for all ages and for the whole area to compare with Vigor's data for the Fenwick district alone*

Yield (in gallons)	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Frequency	1	3	18	33	81	146	275	442	592	752	819	850	769	636

Yield (in gallons)	19	20	21	22	23	24	25	26	27	28	29	30	Total.
Frequency	511	387	239	151	103	63	30	18	8	3	4	1	6,935

Mean=15.991±0.027. Standard deviation=3.329±0.014. Coefficient of variation=20.816±0.088.

4. The difference between this value of the standard deviation and Vigor's, while reduced, is still sensible. It amounts to about 0.742 ± 0.082. This remains to be accounted for. We find it difficult to suppose that the selection of relatively long lactations in the present data can be the cause, since Vigor himself has shown that there is no sensible correlation between either mean weekly yield or fat content and duration of lactation. We are much more inclined to the view, especially in the light of unpublished results on milk production in other breeds of cattle, that the Fenwick district returns give somewhat abnormal values in the

direction of heightened variability and also in certain other respects which need not be gone into here.

5. Comparison of the present results with those of Gavin (*2*, *3*) leads to the same conclusions as those reached in the preceding paragraphs. In Gavin's first paper (*2*), where 1,233 normal lactations of cows of all ages lumped together are discussed, coefficients of variation are given as follows: For total lactation yield 25.72; for average daily yield 25.78; for maximum daily yield 24.68; and for revised maximum daily yield 24.77. These values are of the same order as those from Vigor's data (coefficient of variation about 24.2) and from the total combined distributions (Table V) of the present paper. In a later paper Gavin (*3*) deals with each of the first five lactation periods separately for a group of about 375 cows. From his data we find the weighted mean coefficient of variation for these five lactations to be 17,998, a value sufficiently close to our weighted mean value for single years of age.

6. Turning to the fat percentage, we see that Vigor's values of 3.681 for the mean and 0.323 for the standard deviation are substantially identical with our weighted mean values of 3.738 and 0.330. Pearson (*23*) has also given some reductions for variation in Ayrshire fat percentages, and the present values are again in close accord with his.

7. It may be concluded that the values of the means and variabilities here given represent essentially normal values for Ayrshire cattle. These constants will be of considerable usefulness as time goes on, for purposes of comparison with other breeds and in the study of special problems.

THE COMPARATIVE VARIABILITY OF MILK PRODUCTION

Milk production is essentially a physiological character. It is a matter of some interest and significance to examine the variability of the character in comparison with other physiological characters and also with some that are more strictly morphological, as, for example, bone measurements. Such comparisons may be made through the coefficients of variation. It must, however, always be kept clearly in mind just what a coefficient of variation is; and care must be taken to avoid drawing too sweeping or even entirely unjustified conclusions from comparison of these constants. What the coefficient of variation measures is the percentage which the "scatter", or variation exhibited by a distribution as measured by the standard deviation, is of the mean of the character varying. For some purposes this percentage is meaningless. It is therefore idle to try to force its use for those purposes. It will undoubtedly be presently supplemented by some other constant for the measurement of other aspects of comparative variability. It has, however, a perfectly definite, if limited, meaning. It is a unique constant of any distribution, expressed in abstract units. As such it may be used for purposes of comparison, always remembering that one must be cautious as to the manner in which conclusions drawn from such comparison are stated.

In Table VI are given coefficients of variation for a number of characters for purposes of comparison with milk yield. The coefficients are arranged in order of descending magnitude.

TABLE VI.—*Coefficients of variation for various characters*

Characters.	Coefficient of variation.	Authority.
Number of children per family (New South Wales)	48.41	Powys (25).
Area of comb (domestic fowl)	39.97	Pearl and Pearl (14).
Weight of spleen (English males)	38.21	Greenwood (4).
Size of litter (mouse)	37.50	Weldon (29).
Lambs per birth (sheep)	35.78	Pearl (11).
Dermal sensitivity (English males)	35.70	Pearson (19).
Annual egg production (domestic fowl)	34.21	Pearl and Surface (15).
Size of litter (Poland-China swine)	27.41	Surface (27).
Size of litter (Duroc-Jersey swine)	26.00	Do.
Milk yield (total lactation)	25.78	Gavin (2).
Milk yield (daily average)	25.72	Do.
Fecundity[a] (horse)	24.77	Calculated from data in Pearson (21).
Heart weight (English males)	22.22	Greenwood and Brown (5).
Weight of kidneys (English males)	21.05	Do.
Weight of liver (English males)	20.82	Do.
Swiftness of flow (English males)	19.40	Pearson (19).
Body weight (English males)	18.91	Greenwood and Brown (5).
Revised maximum daily milk yield (for given age)	17.998	Gavin (3).
Weekly milk yield (Ayrshire cattle)	*17.08*	*This paper.*
Breathing capacity (English males)	16.60	Pearson (19).
Strength of pull (English males)	15.00	Do.
Weight of shell of egg (domestic fowl)	13.86	Curtis (1).
Body weight (domestic fowl)	12.66	Do.
Weight of albumen of egg (domestic fowl)	12.27	Do.
Length of red blood corpuscles (Bufo tadpoles)	11.85	Pearson (22).
Weight of yolk of egg (domestic fowl)	11.31	Curtis (1).
Amount of fat in mixed milk (daily fluctuations)	9.68	Pearl (10).
Yield of mixed milk (daily fluctuations)	9.05	Unpublished data in this laboratory.
Weight of egg (domestic fowl)	8.36	Pearl and Surface (16).
Brain weight (Bavarian males)	8.12	Pearl (9).
Length of forearm (English males)	5.24	Pearson and Lee (24).
Length of femur (French males)	5.05	Pearson (19).
Length of egg (domestic fowl)	4.24	Pearl and Surface (16).
Stature (English males)	3.99	Pearson and Lee (24).
Horizontal circumference of skull (English males)	2.87	Macdonell (7).
Specific gravity of egg (domestic fowl)	.50	Pearl and Surface (16).

[a] Fecundity here means the fraction which the actual number of offspring arising from a given number of coverings is of the possible number of offspring under the circumstances.

This table brings out the well-known fact, which has been discussed in some detail by Pearl (9), Gavin (2), and others, that, in general, physiological characters exhibit high coefficients of variation as compared with strictly morphological characters. Characters which are intermediate in their quantitative determination, as, for example, the length of the egg in the domestic fowl, give coefficients of variation intermediate in value. Purely physical characteristics which are usually regarded by physicists and chemists as "constants," such as the specific gravity of eggs, show very low coefficients of variation.

It is of interest to compare the coefficients of variation for total yield and absolute amount of fat in the mixed milk of a large herd with those for milk yield as discussed in the present paper. It is seen that the former are about 9, whereas the coefficients for milk yield give values of about 17 to 25, depending upon whether cows of all ages or of a single age are considered.

In secular variation in the amount or quality of the mixed milk of a large herd, individuality of the animal as a source of variation is entirely

eliminated. The observed variation must, therefore, be due to the combined action of all the external environmental influences which affect in greater or less degree the milk yield of every cow.

On the other hand, the constants of variation for milk yield determined in this paper are based upon the diversity or variation in weekly yield exhibited among a large number of different cows. Here one primary factor in the causation of the observed variation must be the individuality of the animal with respect to milking ability. By individuality in this sense is meant the genotype of the individual with regard to the character named. But in the causation of the variation in milk yield as here discussed there must be involved the combined influence of the individuality of the animal plus that of all the environmental factors which act in producing variation in the mixed milk of the herd, since each of these causes influences every individual animal while it is making its individual record.

It is therefore possible to make comparison here between observed variations (as measured by the coefficient), due, on the one hand, to environmental influences alone and, on the other hand, to genotypic differences plus environmental influences. The difference should represent in a general way that part of the observed variation due to genotypic differences.

The figures as they stand suggest that roughly about one-half of the variation (measured by the coefficients of variation) in milk production results from the varying genotypic individuality of the animals with respect to this character, and the other half results from the varying external circumstances to which cows are subjected during lactation and which have an effect upon the flow of milk. Or, to put the matter in another way, if the conclusion just stated were true it would mean that if a large number of cows were placed in environmental circumstances which were at once ideal and uniform we should expect the variation exhibited in milk production to be roughly about one-half of that which we actually find when we measure this variation under ordinary circumstances.

Another point of interest in connection with Table VI is the comparison of the coefficients of variation for milk yield with that for the weight of the albumen of the egg of the domestic fowl. Both of these are secreted products. The weight of the shell of the egg is another character falling in the same category. The figures here given indicate that the variation in these characters, taken in relation to their respective means, is greater for milk secretion than for albumen or shell secretion. Or, put in another way, the oviduct as a secretory organ appears to work truer to type than does the udder of the cow. This result is what would be expected from all that is known of the physiology of the two organs. The secretory activity of the cow's udder is apparently very much more easily influenced by external circumstances and by nervous impulses than is the oviduct of the fowl.

ANALYTICAL DISCUSSION OF VARIATION IN MILK PRODUCTION

Turning next to the analysis of the variation in mean weekly yield and in fat percentage by fitting skew curves to the observed frequency distributions, we have the results set forth in Tables VII and VIII. Table VII gives the analytical constants for mean weekly yield and Table VIII those for fat percentage. In fitting the curves the combined distributions for the two years 1908 and 1909 have been used throughout in the case of weekly yield. In the case of fat percentage the combined distributions have been used for all ages except 3 and 4 years. Since there was some doubt as to whether, at these ages, the fat distributions for the two years were significantly different from each other, it was thought best to fit the 1908 and the 1909 fat distributions separately for both of the ages mentioned.

TABLE VII.—*Analytical constants for variation in mean weekly yield*

Constant.	3 years.	4 years.	5 years.	6 years.	7 years.
N	1,441	1,118	909	804	712
μ_2	23.9798	30.1002	30.3681	35.0959	35.2890
μ_3	17.7255	21.6903	61.4079	55.2151	80.0331
μ_4	1,830.3431	2,986.7220	3,604.8731	4,201.6107	4,307.9103
β_1	.0228	.0173	.1346	.0705	.1458
$\sqrt{\beta_1}$	.1509	.1313	.3669	.2656	.3818
β_2	3.1830	3.2965	3.9089	3.4112	3.4593
β_2-3	.1830	.2965	.9089	.4112	.4593
κ_1	.2977	.5413	1.4138	.6107	.4813
κ_2	.0578	.0240	.0746	.0883	.2357
Skewness	.0688	.0560	.1302	.1115	.1661
d (gallons)	.1686	.1537	.3587	.3304	.4934
σ (gallons)	2.4485	2.7432	2.7554	2.9621	2.9702
Mean (gallons)	13.8413	15.2299	16.4634	17.4701	18.0492
Mode (gallons)	13.6727	15.0762	16.1047	17.1397	17.5558
Range (gallons.)	.....	.....	.....	.....	.....
+end of range	.....	.....	.....	.....	.....
−end of range	.....	.....	.....	.....	.....
Y_0 per cent	8.28	7.50	7.79	6.99	6.97
P. E. $\sqrt{\beta_1}$	± .0435	± .0494	± .0548	± .0583	± .0619
P. E. β_2	± .0870	± .0988	± .1096	± .1165	± .1238
P. E. skewness	± .0217	± .0247	± .0274	± .0291	± .0309

Constant.	8 years.	9 years.	10 years.	11 years.	12 years.
N	637	459	320	202	116
μ_2	35.2522	33.8404	36.9035	44.3448	38.1075
μ_3	59.3923	48.3269	44.3365	−22.3850	117.4164
μ_4	4,334.0982	3,464.0612	4,387.5182	7,459.8357	4,642.5835
β_1	.0805	.0603	.0391	.0057	.2491
$\sqrt{\beta_1}$	.2838	.2455	.1978	.0758	.4991
β_2	3.4876	3.0249	3.2217	3.7935	3.1970
β_2-3	.4876	.0249	.2217	.7935	.1970
κ_1	.7336	−.1309	.3260	1.5698	−.3535
κ_2	.0842	−.3505	.0909	.0009	−1.1203
Skewness	.1157	.1283	.0895	−.0259	.2817
d (gallons)	.3435	.3732	.2718	−.0863	.8695
σ (gallons)	2.9687	2.9086	3.0374	3.3296	3.0866
Mean (gallons)	18.2602	18.5561	18.7375	18.1114	18.4569
Mode (gallons)	17.9167	18.1829	18.4657	18.1977	17.5874
Range (gallons)	.....	64.4991	.....	.....	45.0305
+end of range	.....	67.2343	.....	.....	54.4776
−end of range	.....	2.7352	.....	.....	9.4471
Y_0 per cent	7.03	6.85	6.57	6.46	6.52
P. E. $\sqrt{\beta_1}$	±.0655	±.0771	±.0924	±.1163	±.1534
P. E. β_2	±.1309	±.1542	±.1847	±.2325	±.3068
P. E. skewness	±.0327	±.0386	±.0462	±.0581	±.0767

In the graduating of the observational data Pearson's (*18*, *20*) skew frequency curves and his method of moments have been used throughout. In the tables the moment coefficients are given in terms of units of grouping.

Only the distributions for the ages 3 to 12 years, inclusive, have been subjected to analytical treatment. Outside of these limits the numbers involved become so small as to make the discussion of them from the point of view of mean weekly yield and fat percentage not worth the labor involved.

TABLE VIII.—*Constants for variation in fat percentage*

Constant.	3 years.		4 years.		5 years.	6 years.
	1908.	1909.	1908.	1909.		
N	614	825	526	591	909	804
μ_2	14.7683	11.7975	12.8584	10.6362	11.9935	10.1970
μ_3	21.1714	11.6005	19.9750	5.8796	10.5807	7.6514
μ_4	734.3389	434.5862	582.6387	342.3461	460.8242	330.2351
β_1	.1392	.0820	.1887	.0287	.0649	.0552
$\sqrt{\beta_1}$	.3730	.2863	.4332	.1695	.2548	.2350
β_2	3.3669	3.1225	3.5239	3.2036	3.2036	3.1760
β_2-3	.3669	.1225	.5239	.0262	.2036	.1760
κ_1	.3164	— .0009	.4848	— .0338	.2126	.1863
κ_2	.3416	— 6.8108	.3044	— .6413	.2327	.2254
Skewness	.1697	.1432	.1886	.0857	.1192	.1108
d (percentage)	.0652	.0492	.0676	.0280	.0413	.0354
σ (percentage)	.3843	.3435	.3586	.3261	.3463	.3193
Mean (percentage)	3.9151	3.8947	3.7696	3.7804	3.7160	3.6852
Mode (percentage)	3.8499	3.8455	3.7020	3.7524	3.6747	3.6498
Range (percentage	.....	.....	.....	15.7482	.....	.....
+ End of range	.....	.....	.....	16.5764	.....	.....
— End of range	.....	1.4607	.....	.8283	.....	.....
Y_0 percent	10.6700	11.6700	11.0700	12.2400	11.7500	12.7000
P. E. $\sqrt{\beta_1}$	± .0667	± .0575	± .0721	± .0680	± .0548	± .0583
P. E. β_2	± .1334	± .1150	± .1441	± .1359	± .1096	± .1165
P. E. Skewness	± .0333	± .0288	± .0360	± .0340	± .0274	± .0291

Constant.	7 years.	8 years.	9 years.	10 years.	11 years.	12 years.
N	712	637	459	320	202	116
μ_2	9.4598	9.9688	9.1889	8.4448	9.5987	10.6494
μ_3	5.0193	9.8014	4.8556	7.5919	9.1894	10.0965
μ_4	244.7592	309.5716	247.6547	228.2603	250.6424	361.6011
β_1	.0298	.0970	.0304	.0957	.0955	.0844
$\sqrt{\beta_1}$	.1725	.3114	.1743	.3094	.3090	.2905
β_2	2.7400	3.1151	2.9330	3.2008	2.7204	3.1885
β_2-3	— .2600	.1151	— .0670	.2008	— .2796	.1885
κ_1	— .6093	— .0607	— .2251	.1144	— .8457	.1237
κ_2	— .0370	— 1.2270	— .1021	.6425	— .0872	.5225
Skewness	.1071	.1589	.0943	.1492	.2194	.1397
d (percentage)	.0330	.0502	.0286	.0434	.0680	.0456
σ (percentage)	.3076	.3157	.3031	.2906	.3098	.3263
Mean (percentage)	3.6906	3.6637	3.6362	3.6003	3.6292	3.5991
Mode (percentage)	3.6576	3.6136	3.6076	3.5569	3.5612	3.5535
Range	2.6461	13.3421	4.5770	.....	2.7333	.....
+ End of range	5.2643	15.2865	6.6211	.....	4.9959	.....
— End of range	2.6182	1.9445	2.0441	.....	2.2625	.....
Y_0 per cent	12.2600	12.7600	13.0100	13.9500	12.3700	12.4200
P. E. $\sqrt{\beta_1}$	± .0619	± .0655	± .0771	± .0924	± .1163	± .1534
P. E. β_2	± .1237	± .1309	± .1542	± .1847	± .2325	± .3068
P. E. Skewness	± .0309	± .0327	± .0386	± .0462	± .0581	± .0767

The histograms and their fitted curves are shown in figures 1 to 4.

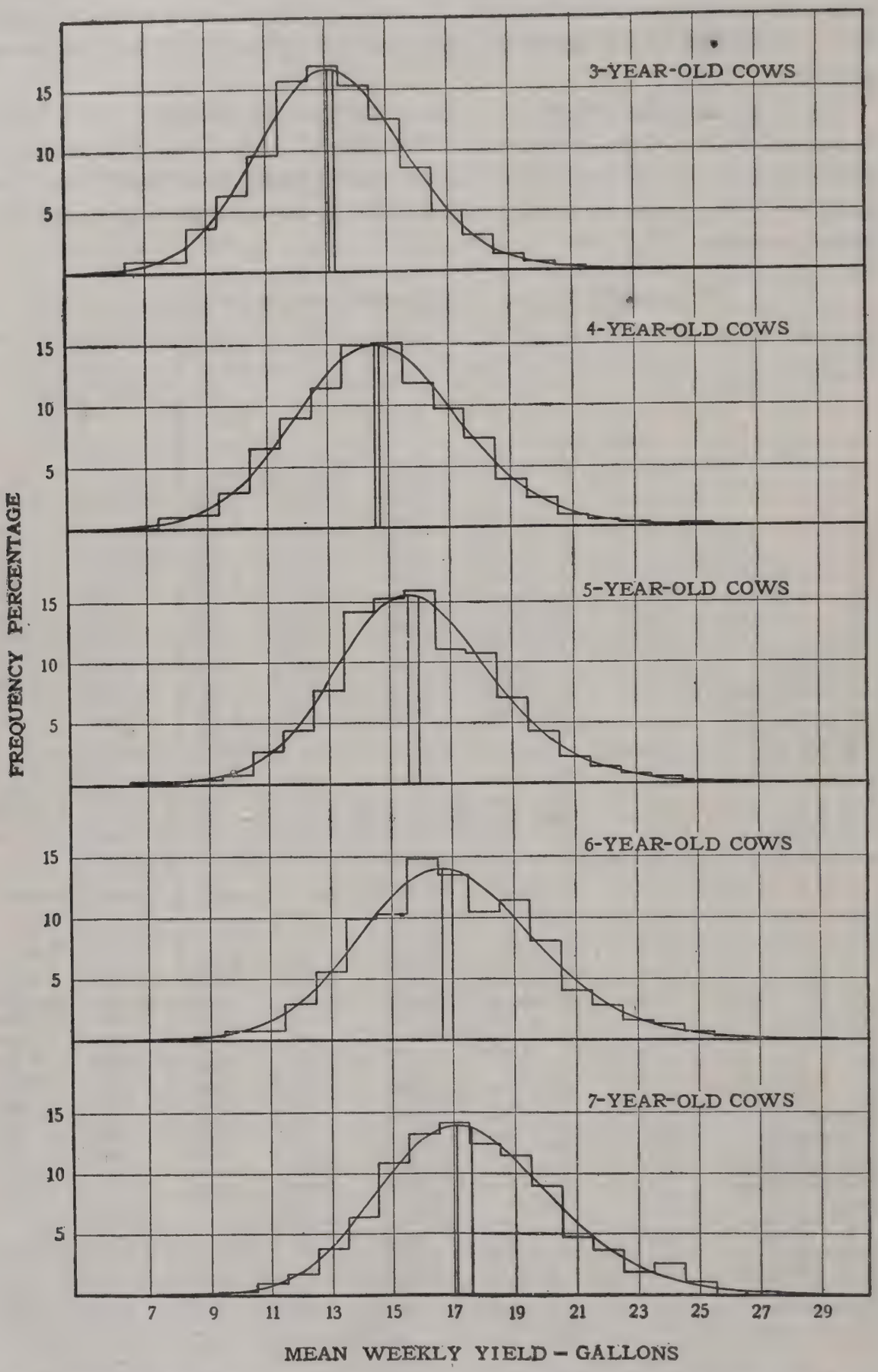

FIG. 1.—Histograms and fitted curves for variation in mean weekly milk yield of Ayrshire cows of ages 3 to 7 years. The ordinates are plotted on a percentage basis, and since the base unit (1 gallon) is the same for all diagrams the areas of all are equal.

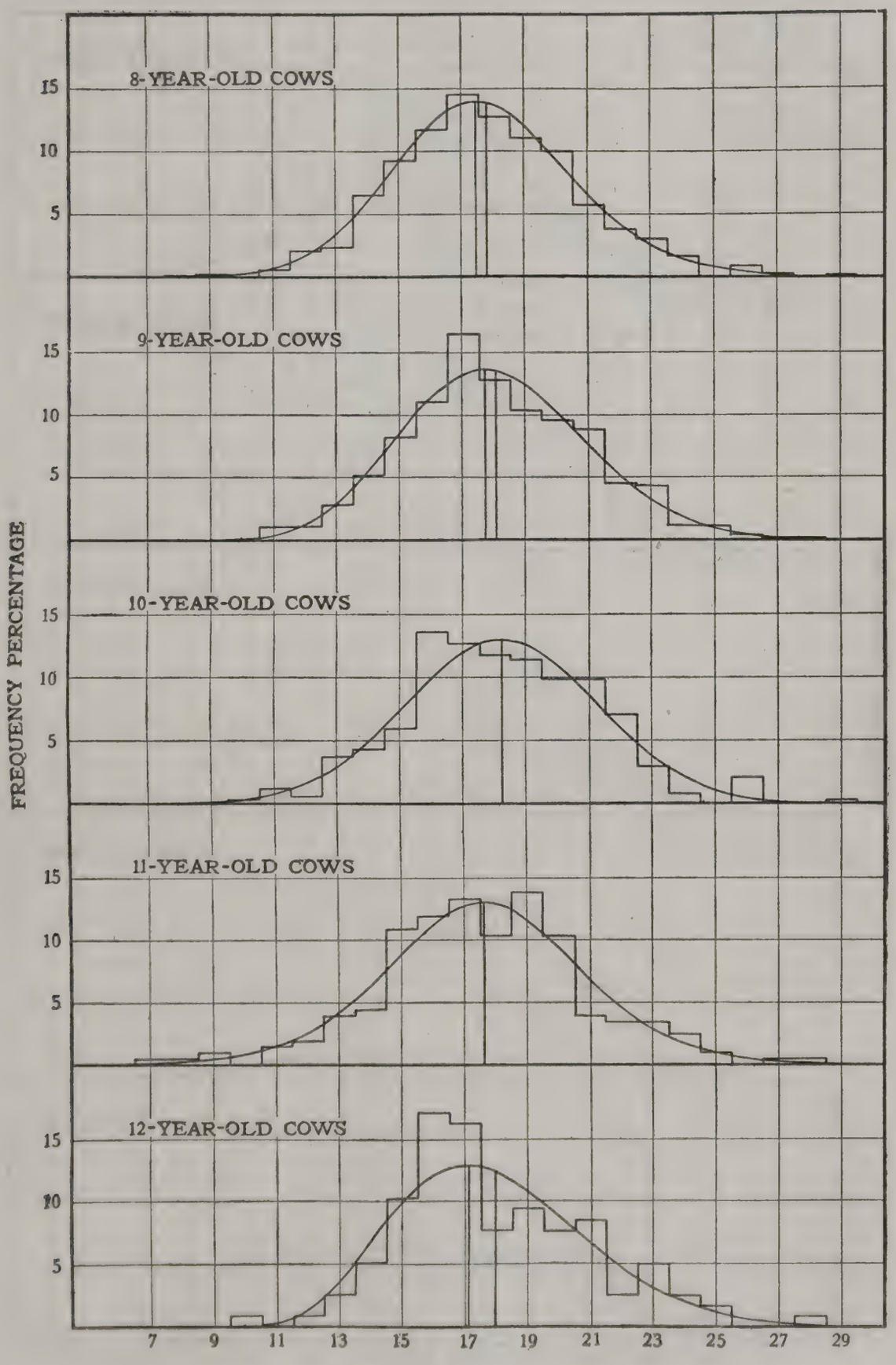

FIG. 2.—Histograms and fitted curves for variation in mean weekly milk yield of Ayrshire cows of ages 8 to 12 years.

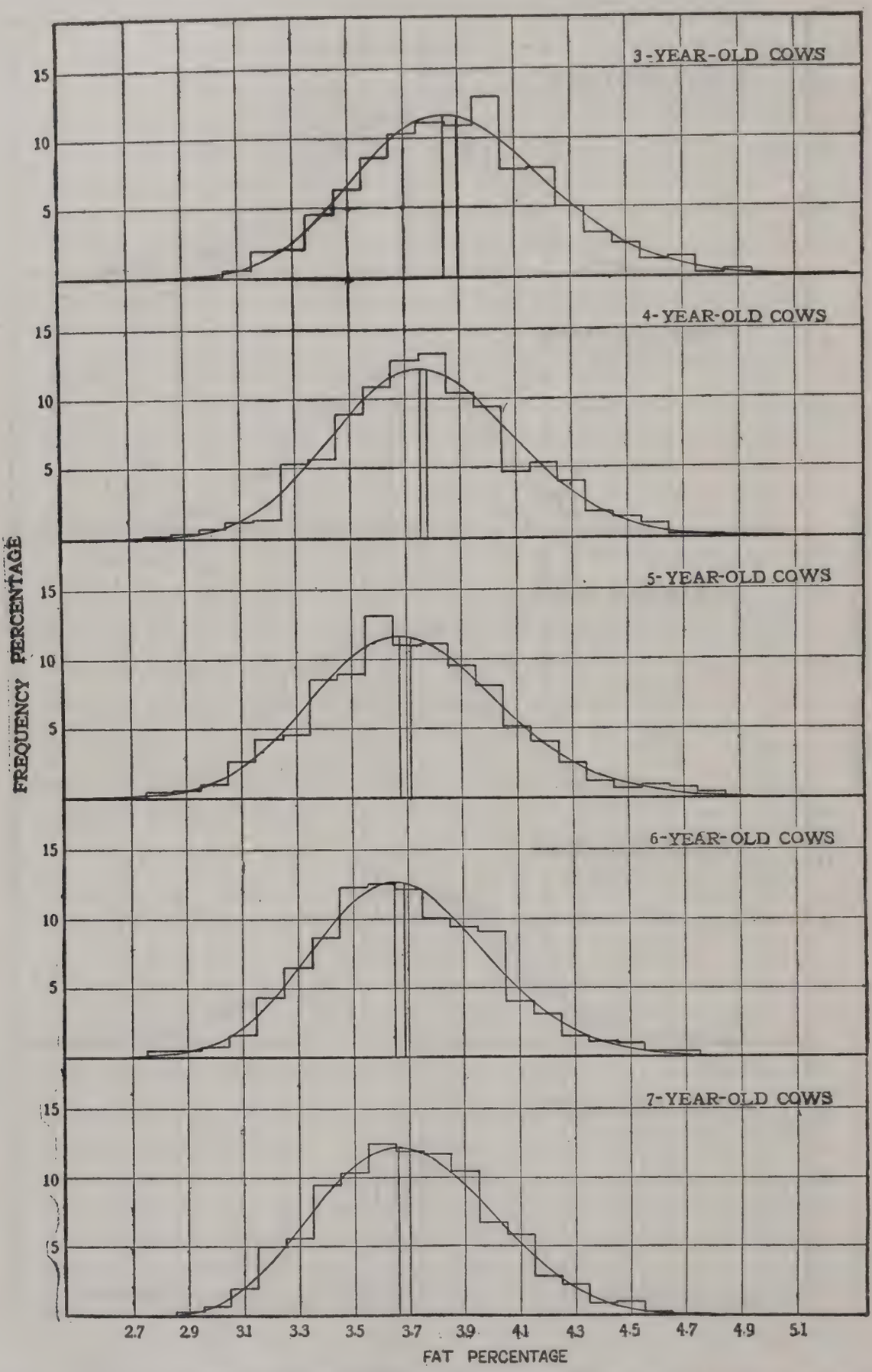

FIG. 3.—Histograms and fitted curves for variation in fat percentage of milk of Ayrshire cows of ages 3 to 7 years. For purposes of illustration the 1909 curves are used in the 3- and 4-year classes.

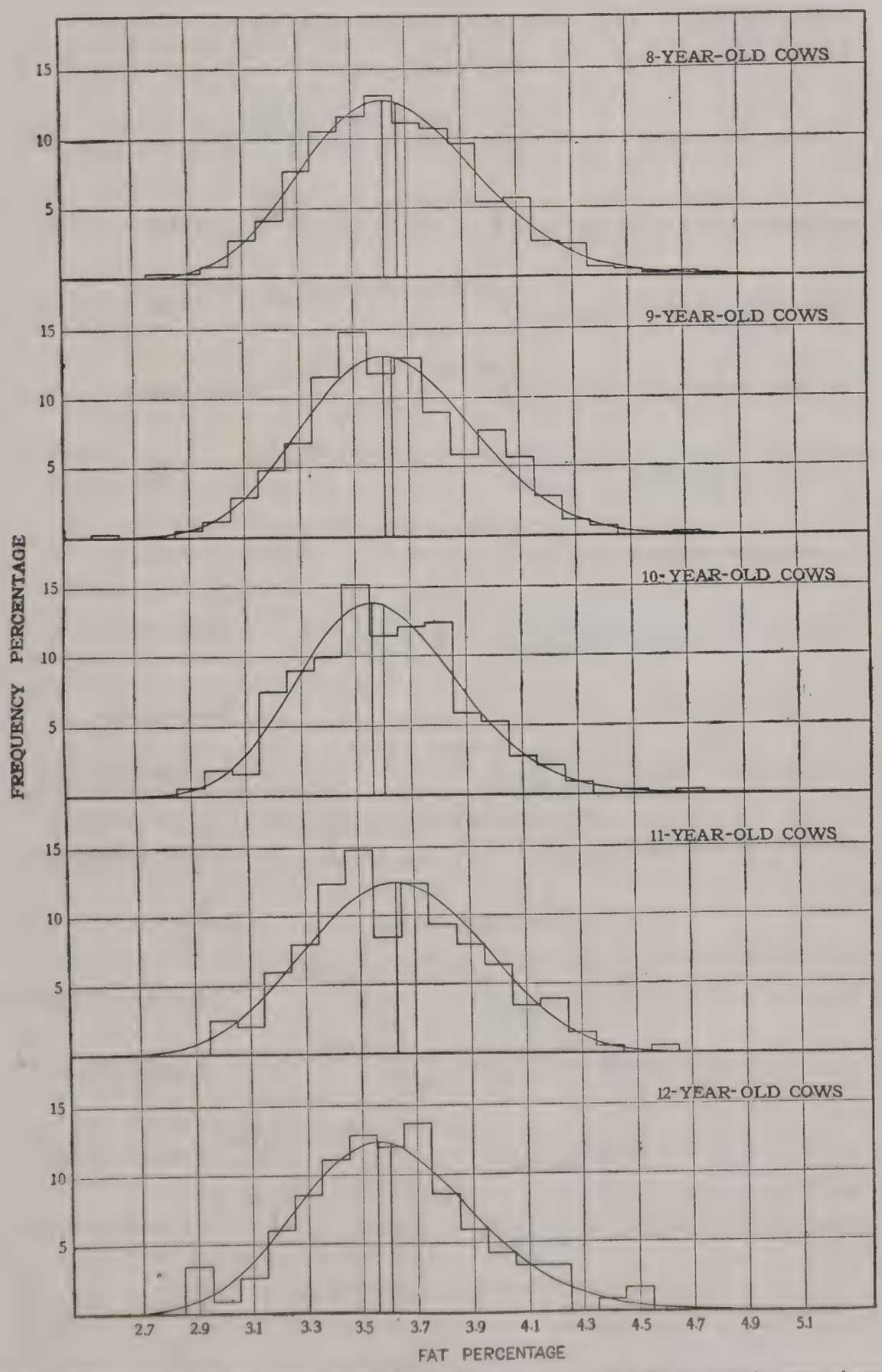

FIG. 4.—Histograms and fitted curves for variation in fat percentage of milk of Ayrshire cows of ages 8 to 12 years.

The equations to the various curves are as follows:

Mean weekly yield

3 years. . $y=33.7848\left(1+\frac{x^2}{961.1385}\right)^{-22.7692}e^{10.7802\tan^{-1}\frac{x}{31.0022}}$ Pearson's Type IV.

4 years. . $y=52.7759\left(1+\frac{x^2}{712.8085}\right)^{-13.6324}e^{3.9661\tan^{-1}\frac{x}{26.6985}}$ Do.

5 years. . $y=47.406\left(1+\frac{x^2}{302.7514}\right)^{-6.8866}e^{3.3430\tan^{-1}\frac{x}{17.3998}}$ Do.

6 years. . $y=20.457\left(1+\frac{x^2}{703.7331}\right)^{-12.4973}e^{7.1576\tan^{-1}\frac{x}{26.5280}}$ Do.

7 years. . $y=0.9198\left(1+\frac{x^2}{750.8586}\right)^{-15.4198}e^{16.0162\tan^{-1}\frac{x}{27.4018}}$ Do.

8 years. . $y=19.871\left(1+\frac{x^2}{603.2364}\right)^{-10.8431}e^{5.9709\tan^{-1}\frac{x}{24.5609}}$ Do.

9 years. . $y=31.4278\left(1+\frac{x}{30.8954}\right)^{21.0838}\left(1-\frac{x}{98.1027}\right)^{66.9477}$ Pearson's Type I.

10 years. . $y=21.0148\,e^{-\frac{x^2}{73.8071}}$ Normal curve.

11 years. . $y=13.0538\left(1+\frac{x^2}{423.9832}\right)^{-6.2805}$ Pearson's Type II.

12 years. . $y=7.5643\left(1+\frac{x}{16.2806}\right)^{5.6158}\left(1-\frac{x}{73.7804}\right)^{25.4498}$ Pearson's Type I.

Fat percentage

3 years, 1908. . $y=0.0038\left(1+\frac{x^2}{401.0906}\right)^{-22.1236}e^{30.4278\tan^{-1}\frac{x}{20.0272}}$ Pearson's Type IV.

3 years, 1909. . $y=96.2952\,e^{-2.0483x}\left(1+\frac{x}{23.8476}\right)^{48.8478}$ Pearson's Type III.

4 years, 1908. . $y=0.2183\left(1+\frac{x^2}{249.6647}\right)^{-15.4559}e^{19.1242\tan^{-1}\frac{x}{15.8008}}$ Pearson's Type IV.

4 years, 1909. . $y=72.3605\left(1+\frac{x}{29.2413}\right)^{65.3887}\left(1-\frac{x}{128.2405}\right)^{286.7682}$ Pearson's Type I.

5 years. . $y=0.0221\left(1+\frac{x^2}{546.3249}\right)^{-31.1847}e^{33.2496\tan^{-1}\frac{x}{23.3736}}$ Pearson's Type IV.

6 years. . $y=0.0098\left(1+\frac{x^2}{531.5363}\right)^{-35.1463}e^{36.8364\tan^{-1}\frac{x}{23.0551}}$ Pearson's Type IV.

7 years. . $y=87.2637\left(1+\frac{x}{10.3942}\right)^{5.8303}\left(1-\frac{x}{16.0669}\right)^{9.0123}$ Pearson's Type I.

8 years. . $y=81.3119\left(1+\frac{x}{16.6913}\right)^{24.7018}\left(1-\frac{x}{116.7294}\right)^{172.7511}$ Pearson's Type I.

9 years. . $y=59.7382\left(1+\frac{x}{15.6350}\right)^{16.6417}\left(1-\frac{x}{30.1351}\right)^{32.0755}$ Do.

10 years. . $y=9.6682\times 10^{-34}\left(1+\frac{x^2}{330.3039}\right)^{-56.1987} e^{147.9872\tan^{-1}\frac{x}{18.1743}}$ Pearson's Type IV.

11 years. . $y=24.9882\left(1-\frac{x^2}{186.7756}\right)^{8.2292}$ Pearson's Type II.

12 years. . $y=2.5479\times 10^{-20}\left(1+\frac{x^2}{513.7690}\right)^{-52.0148} e^{106.7238\tan^{-1}\frac{x}{22.6665}}$ Pearson's Type IV.

From Tables VII and VIII and the accompanying curves the following points are to be noted:

1. It is apparent that the fitted curves give very good graduations of the data throughout. Pearson's generalized probability curve has been shown by experience to be applicable in one or another of its types to so wide a range of cases that a new application calls for no special mention. However the continued addition of new classes of data easily and perfectly graduated by these curves is the best refutation of the criticisms which were formerly made against them.

2. The general tendency of these milk production and fat percentage variation curves is plainly toward positive skewness. All of these curves show a positive skewness, with the single exception of the mean weekly yield curve for cows 11 years old. There the skewness is minus but in comparison with its positive error (on the basis of the normal curve) is insignificant. In other words, this curve for 11-year-old cows is, within the limits of error of random sampling, a symmetrical distribution. All the others are skew in the positive direction, or, in other words, the mean is greater than the mode.

3. Considering the probable error of this skewness on the basis of a normal curve, it is seen that in 7 out of the 10 curves for the mean weekly yield the skewness is three or more times its probable error. In 2 cases it is somewhat less than three times its probable error, while in 1 case the skewness is certainly insignificant—that for 11 years, as already noted. In the case of fat content 9 out of the 11 curves show a skewness three or more times the probable error. In 2 of the remaining cases the skewness is nearly three times the probable error, while in 1 case—that of the 12-year-old cows where the number of individuals concerned is small—the skewness is distinctly less than three times its probable error. From these figures it is plain that in general these Ayrshire milk variation curves show a significant tendency toward an asymmetry indicated by a positive skewness.

4. It is of some interest to examine the weighted mean value of the skewness for all the curves, the weighting being in proportion to the number of individuals involved, in comparison with the skewness exhibited in the variation curves of other characters. We have for the weighted mean value of the skewness for mean weekly yield, the 11-year curve being omitted, a value of +0.1047. For the variation curves for fat content the weighted mean value of the skewness is +0.1338. It was shown by Pearl and Surface (*15*) that in variation in annual egg production in Barred Plymouth Rocks the skewness is always negative and usually significant. This difference in skewness between the two characters milk production and egg production is striking. Curves of variation in egg production tail off more on the side toward low egg production, whereas the curves of variation in milk production tail off more on the side toward high production. The weighted mean values of the skewness for annual egg production in three successive years were found to be −0.280, −0.122, and −0.108. In other words, the values in general were of an order of magnitude not far from that here found for the skewness of curves of variation in milk production.

5. It might at first thought be supposed that the direction of the skewness in milk productive curves was due to selection—that is, to the continued culling out of the poor producers. Since, however, the same factor of selection in the direction of the high producers was operative to as great or even a greater extent in the making up of the flocks from which the annual egg production variation curves were obtained, it seems perfectly clear that selection can have had very little to do with bringing about the difference in direction of skewness exhibited by egg and milk production curves respectively. The inference would then seem strongly justified that selection had nothing to do with the production of the asymmetry of the variation curves in either case considered by itself.

6. Additional interest is given the matter when an examination is made of the facts regarding the direction of the skewness in the variation of the hen's egg in size characteristics. Such data have been furnished by Pearl and Surface (*16*). They show (*p. 184*) that in the variation of egg length, egg bulk, egg weight, and egg breadth the skewness is positive and significant in all cases except that of breadth. We see here again emphasized a point which comes out frequently in biometrical work—namely, that there is frequently between characters a parallelism in variation corresponding to a parallelism in the underlying physiological bases of the characters. This relation is clearly apparent in the present instance. The size of the egg is primarily determined by the secretory activities of the oviduct. It is a character which is physiologically much more directly comparable to milk production than is total annual production. Primarily the latter depends physiologically upon quite another thing—namely, the

inherited genes for fecundity which determine the frequency and regularity of ovulation. Corresponding to the physiological parallelism in egg size and milk production is found corresponding asymmetry of the variation curves, as well as a closer relationship between other of the variation constants in the two cases than is found when milk production is compared with egg yield.

7. Considering the types of the curves, we find that 7 out of 10 curves for mean weekly yield give upon analysis unlimited range curves—in 6 cases the skew Type IV and in one case the symmetrical normal curve. Something approaching the reverse condition is found with respect to variation in fat percentage. Five out of the 12 distributions for this character lead upon analysis to curves with the range limited at both ends (Type I) and one to a curve of Type III, which is limited at the lower range end. The remainder of the curves are of Type IV, but near the border line of passage over to the limited range types. It would then appear that the physiological fact that variation in percentage of fat content will necessarily tend to be confined within relatively narrower limits than variation in total flow of milk is reflected in the distribution of the several curves in respect to type.

8. The estimation of the range ends in the case of the limited range curves is on the whole fairly good, leading in no case to absolutely impossible values regarding the probable errors involved. The determination of the range ends in the Type I curve is subject to rather considerable probable errors. The most extreme range end estimation in mean weekly yield is that given by the curves for 9-year-old cows. This gives for the upper range end 67.2 gallons a week. This of course would be an extraordinarily high average weekly yield, yet it probably can not be regarded as physiologically impossible. It certainly would not be for a single week. Indeed such a record is rather frequently exceeded by Holstein-Friesian cows which on official tests may occasionally go to a production over 100 gallons per week. In fat percentage the most extreme range estimation is for the 1909 curve for 4-year-old cows, which gives for the upper end of the range 16.6 per cent fat. Again this figure, while of course extraordinary for an average test, probably indicates no physiological impossibility for brief periods of time. That such is the fact is indicated by some of the short period tests of Jersey cows.

9. In all of the curves for mean weekly yield the kurtosis is positive. In other words, these curves show a tendency of greater or less degree toward the leptokurtic condition. They are more peaked than would be normal curves of corresponding standard deviations. The value of the kurtosis is probably significant in all the mean weekly yield curves, with the exception of those for 3-year-old, 9-year-old, 10-year-old, and 12-year-old animals. In curves of variation in fat percentage there is no such uniform tendency in regard to the value of the kurtosis. The

1908 3-year-old curve is probably significantly leptokurtic. The 1909 3-year-old curve does not appear to differ significantly from the normal in this respect. The same relations hold in regard to the 1908 and 1909 4-year-old curves. The 5-year-old distribution is distinctly leptokurtic. The 6-year-old distribution is probably mesokurtic. The 7-year-old distribution is probably platykurtic. This is the first of the fat curves to give a negative value for the kurtosis. The remainder of the curves are significantly mesokurtic.

CAN THE VARIATION IN MEAN WEEKLY YIELD BE BETTER REPRESENTED BY THE SUM OF TWO NORMAL CURVES OR BY A UNIMODAL SKEW FREQUENCY CURVE?

An examination of certain of the raw distributions for variation in milk yield suggested that possibly we were dealing here with bimodal distributions. Such a possibility is well worth testing thoroughly on theoretical grounds, since if it were found that milk production curves were bimodal this fact might be used as a first point of departure in the determination of the number and characteristics of the (presumably multiple) genes concerned in the inheritance of this character. We have consequently subjected certain of the distributions to the method of analytical dissection discovered by Pearson (*17*).

The distribution chosen for dissection were those for 5- and 6-year-old cows, the combined distribution for the two years (1908 and 1909) being used in both instances. (Compare Table I.)

It will not be necessary here to go over all the details of the laborious arithmetic involved in this work. It will suffice to show, as is done in Table IX, the best solutions when these two distributions are regarded as the sum of two normal curves in each case.

TABLE IX.—*Constants of the component normal curves in the variation in mean weekly yield*

Constant.	5-year-old cows.		6-year-old cows.	
	First component.	Second component.	First component.	Second component.
Area	716.900	192.100	496.600	307.400
Mean (gallons)	16.115	17.764	16.890	18.408
S. D. (gallons)	2.298	3.752	2.418	3.476
Modal ordinate	62.220	10.210	40.970	17.640

From this table it is seen that the dissection gives in both cases a lower component curve of large area and small standard deviation and an upper component of smaller area and much larger standard deviation. This is exactly the sort of result which might well be expected if milk yield depended upon two hereditary factors, the higher one of which was linked with sex or some other factor.

The graduation obtained by the summing of two curves was, in general, a good one. But before drawing any conclusions regarding genetic factors from these successful resolutions of the variation curves into two components it will be well to determine quantitatively, by means of Pearson's test for goodness of fit, whether the two component curves or the unimodal skew curves give the better graduations. Carrying out this test the following values are found:

	Skew curve.	2-component curve.
5-year-old cows	P=0.774	P=0.717
6-year-old cows	P=0.624	P=0.599

It thus appears that while both the skew curves and the 2-component curves graduate this material rather well, there is a distinct, if not large, advantage with the skew curve in each case.

To sum the whole matter up it may be said that, while it is possible to graduate milk production variation distributions as the sum of two normal curves, the resulting fit is not so good as that obtained with the appropriate skew frequency curves. There is no evidence from the analysis of the variation curves to indicate either that milk production distributions are bimodal or that this character depends upon two rather than some other number of genetic factors.

THE RELATION OF MILK AND FAT PRODUCTION TO AGE

With the analyzed variation data in hand it is possible now to consider the problem of the changes in milk production per unit of time and in mean fat percentage, with advancing age of the cow. The great importance of a thorough and comprehensive knowledge of these relationships, if one is to make any adequate investigation of the inheritance of milk and fat production, is sufficiently obvious. It is a perfectly well-known fact, incorporated in all rules for advanced registry of dairy cattle, that milk production does change with age, and to a marked degree. Until investigations on this subject were undertaken in the Biological Laboratory of the Maine Station some years ago it has always been assumed by those (such as advanced registry officials) who have had to deal with the problems that the changes of milk production with age were linear up to "mature" age, usually taken as 5 years, and that after that time there was no further change with advancing age. How far wrong such an assumption is will be shown graphically below. It was pointed out two years ago by Pearl (*12*) in a preliminary paper based on calculations then completed that the fundamental law of change with milk flow with age is logarithmic.

Let us now examine the facts for Ayrshires, considering first mean weekly yield. The necessary data are given in Table III. The mean

weekly yields in gallons for the combined distributions from age 2 to age 16, inclusive, are exhibited graphically in figure 5. The zigzag line gives the observed production as ordinate against age as abscissa. The smooth curve is a logarithmic curve of which the equation is

$$y = 12.4766 + 0.6146x - 0.0366x^2 + 3.6641 \log x,$$

where y denotes mean weekly yield in gallons and x age in years, taking origin from 1 year. This curve was fitted by the method of moments (compare Miner *8*).

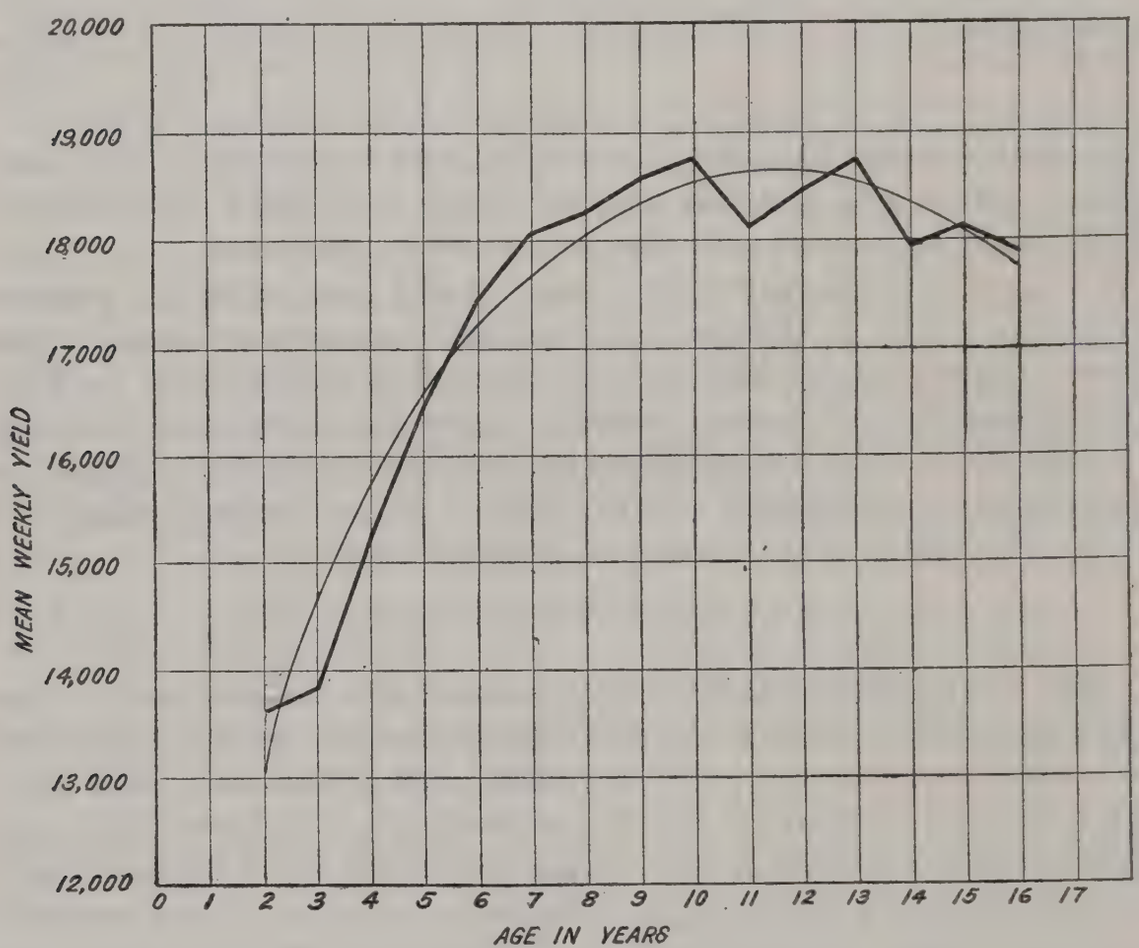

FIG. 5.—Showing the change in mean weekly yield of milk in Ayrshire cows. The smooth curve is of the form $y=a+bx+cx^2+d \log x$.

The actual figures, observed and calculated, are given in Table X.

It is evident from Table X and the diagram that the change here is logarithmic. No better agreement between observation and theory than that here shown could be expected. The law of change may be stated in words in the following way: In these Ayrshire cattle the absolute amount of milk produced per unit of time increases with the age of the cow until a maximum is reached, but the rate of increase diminishes with advancing age until the absolute maximum of production is reached. After the time of maximum productivity the absolute production per unit of time decreases with advancing age, and at a continually increasing rate. This conclusion agrees with that of Pearl and Patterson (*13*) for Jerseys.

TABLE X.—*Comparison of observed mean weekly yields at different ages with those calculated on the assumption that the change is logarithmic*

Age (in years).	Mean weekly yield (in gallons).		Age (in years).	Mean weekly yield (in gallons).	
	Observed.	Calculated.		Observed.	Calculated.
2	13.610	13.055	10	18.738	18.524
3	13.841	14.656	11	18.111	18.610
4	15.230	15.730	12	18.457	18.608
5	16.463	16.544	13	18.750	18.519
6	17.470	17.183	14	17.950	18.346
7	18.049	17.684	15	18.131	18.091
8	18.260	18.067	16	17.875	17.754
9	18.556	18.344			

With the equation relating to mean weekly yield and age in hand we may consider the important problem of the age at which milk production is at a maximum in these cows. To get an answer to this question we have obviously only to equate $\frac{dy}{dx}$ to zero and solve for x.

We have

$$\frac{dy}{dx}=0.6146-0.0732x+\frac{1.5913}{x}.$$

When $\frac{dy}{dx}=0$, we have

$$x=10.4720.$$

Or, we may say that in the large group of cows here dealt with the maximum rate of milk production per unit of time is reached only when the cow is 10½ years old.

Turning next to the relation of fat percentage to age, we have the essential data exhibited in Table XI, the values being taken from Table IV.

TABLE XI.—*Mean fat percentage at different ages*

Age (in years).	Mean fat percentage.		Age (in years).	Mean fat percentage.	
	Observed.	Calculated.		Observed.	Calculated.
2	3.852	3.862	10	3.600	3.607
3	3.903	3.827	11	3.629	3.604
4	3.775	3.793	12	3.599	3.601
5	3.716	3.759	13	3.606	3.598
6	3.685	3.725	14	3.592	3.595
7	3.691	3.690	15	3.500	3.593
8	3.664	3.656	16	3.662	3.590
9	3.636	3.622			

From an examination of the observed figures it appears that in general the fat percentage tends to decline with advancing age until the tenth

year is reached. From that point on, allowing for chance fluctuations and the fact that the numbers dealt with get progressively smaller, the fat percentage appears to remain about constant for the rest of the cow's milking life. Consequently, it has seemed best to break the curve at the 10-year point and fit the two parts separately, each with a straight line. The resulting figures are given in the "calculated" column of Table XI, and are shown graphically in figure 6. The equations to the two lines

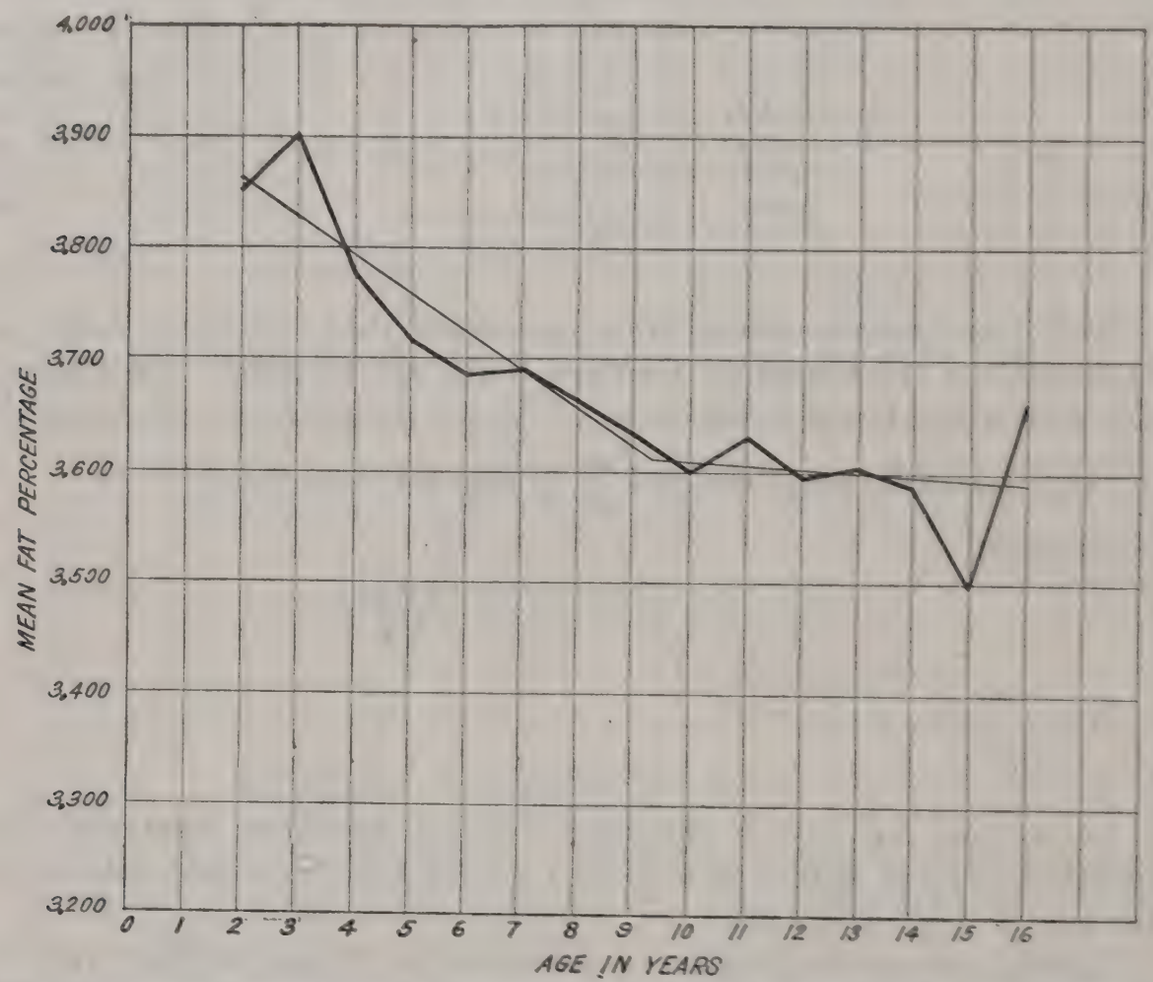

FIG. 6.—Showing the observed (zigzag line) and calculated (straight line) changes in the mean fat percentage of the milk of Ayrshire cows with advancing age.

are as follows, the fitting having been done by the method of least squares.

From 2 to 10 years of age:

$$y = 3.896 - 0.0343x.$$

From 10 to 16 years of age:

$$y = 3.610 - 0.0028x.$$

SUMMARY

This paper presents the results of a biometrical analysis of variation in the quantity per unit of time, and the quality, as indicated by fat percentage, of the milk of Ayrshire cows. Its purpose is to establish normal constants for interindividual variation in these characters, to serve as a base of reference in future genetic studies on milk production.

The chief results of this first part of the investigation may be summarized as follows:

(1) The mean weekly yield and fat percentage of the milk change in a considerable degree and definite manner with increasing age of the cow.

(2) The weighted mean standard deviation and coefficient of variability for mean weekly yield of cows of any given age are 2.806 gallons and 17.081 per cent respectively. Reasons are given tending to show that these may be taken as very close approximations to true normal values. For cows of all ages lumped together the corresponding values are 3.329 gallons and 20.816 per cent.

(3) For fat percentage the weighted mean values for cows of any given age are as follows: Mean = 3.732, standard deviation = 0.330, and coefficient of variation = 8.827.

(4) A table is presented (p. 18) showing the relative variability of milk production as compared with other physiological characters. The udder as a secreting organ is compared with the oviduct of the hen; and it is shown that the oviduct considered as a mechanism operates with somewhat less variability than does the udder, having regard to the absolute weight of the product in the two cases.

(5) Evidence is presented which indicates that about one-half of the observed variation in milk production results from the varying genotypic individuality of the animals with respect to this character and that the other half results from varying environmental influences.

(6) Milk production curves, analytically considered, tend definitely toward positive skewness. This is true in respect to yield and to quality. The weighted mean value of the skewness for mean weekly yield is found to be +0.1047, and that for fat percentage +0.1138.

(7) Evidence is presented which indicates that selection can have had little if anything to do with determining the direction or the amount of skewness shown by milk production curves.

(8) The curves for milk yield tend on the whole to fall more frequently in unlimited range types, while those for fat percentage tend more to limited range types. The estimation of range ends given by the theoretical curves are, on the whole, good.

(9) In general the tendency of milk yield curves is toward the leptokurtic condition—that is, they are more peaked than the corresponding normal curves would be. Fat percentage curves do not show any definite tendency with respect to kurtosis.

(10) Certain of the milk yield curves were dissected into two normal curves by Pearson's method. The resulting graduation was not so good as that given by the appropriate unimodal skew frequency curve. There is no evidence that variation curves for milk production curves are bimodal.

(11) The change in mean weekly yield of milk with advancing age is found to be represented by a logarithmic curve, and to be in accordance

with a law which may be stated in this way: The absolute amount of milk produced per unit of time increases with the age of the cow until the maximum is reached, but the rate of increase diminishes with advancing age until the absolute maximum of production is reached. After the time of maximum productivity, the absolute production per unit of time decreases with advancing age at a continually increasing rate.

(12) The mean fat percentage of the milk was found to decline with advancing age until the tenth year of the cow's life is reached. From that point on, the fat percentage remains about constant through the remainder of the milking life of the cow.

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All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

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ERRATA AND AUTHORS' EMENDATIONS

Page 82, Table II, "Gm." should read "Pounds."

Page 158, line 2, "variability" should read "viability."

Page 160, line 23, "0.183" should read "—0.183."

Page 162, Table VII, "Months required for maturity" should stand above the table. The legend at the left of the table should be "Month in which fruit set."

Page 179, line 30, "petroleum, ether" should read "petroleum ether."

ILLUSTRATIONS

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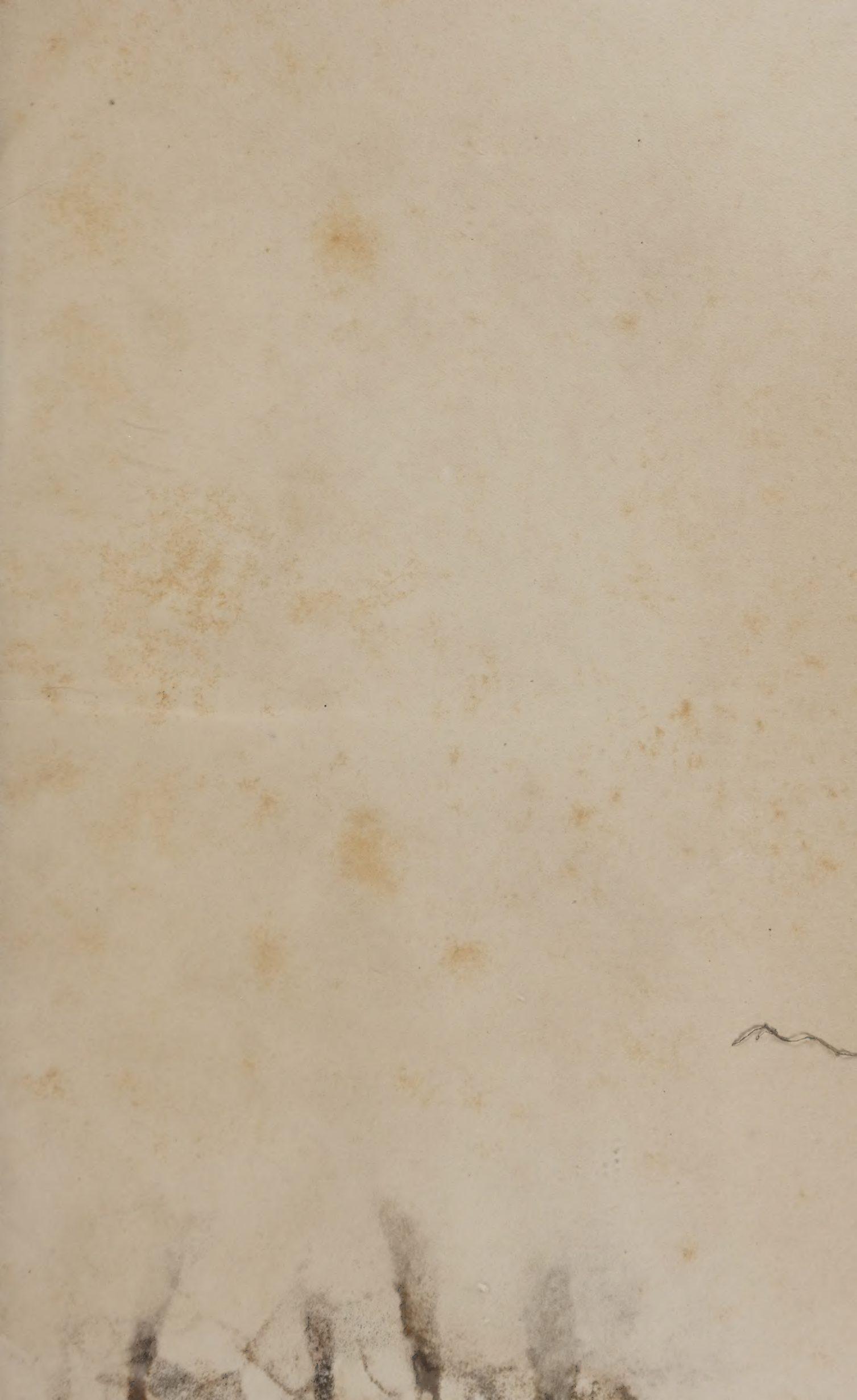
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